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Genetic Diversity, Pollination Ecology and Organoleptic Characteristics of *Coffea arabica* L. in Ethiopian Moist Forests of Different Management Intensity

Gezahegn Berecha Yadessa

Supervisors:

Promotor: Prof. Dr. ir. Olivier Honnay

Co-promoter: Prof. Dr. Isabel Roldán-Ruiz

Co-promoter: Dr. ir. Raf Aerts

Members of the Examination Committee:

Prof. Dr. ir. Hans Jacquemyn, Chairman

Prof. Dr. ir. Bart Muys

Prof. Dr. ir. Wannes Keulemans

Prof. Dr. Luc Lens

Dr. ir. Katrien Vandepitte

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SUMMARY

Coffea arabica L., the world most important commercial coffee species, has its center of origin and diversity in the Afromontane rainforests of southwestern Ethiopia. These forests, which harbour the most important *C. arabica* gene pool, are threatened by increasing anthropogenic forest fragmentation and degradation, and forest management for coffee cultivation. In SW Ethiopia, forest management intensities for coffee cultivation range from almost no intervention in the 'forest coffee' (FC) system to far-reaching interventions that include the removal of competing shrubs and selective thinning of the upper canopy in the 'semi-forest coffee' (SFC) system. Besides, farmers introduce seedlings from wild genotypes or genotypes from locally improved coffee berry disease resistant cultivars in the highly managed semi-forest coffee systems in order to boost coffee productivity.

To study these potential effects we formulated the following objectives. First we assessed the extent of within and among population genetic diversity and the introgression risk of introducing distant landraces and improved cultivars of wild Arabica coffee in SW Ethiopian rainforests along a forest management intensity gradient. Next, we quantified the abundance and diversity of pollinators of wild coffee and mating patterns variation in wild *C. arabica* populations along a gradient of increasing fragmentation and management. Then, we investigated the effect of forest management intensity on pollen limitation and fruit set in wild *C. arabica* populations. Lastly, we quantified the effects of both forest management intensity and forest fragmentation on the organoleptic quality of Arabica coffee. We approached these objectives by genotyping considerable number of populations from highly managed and unmanaged forest fragments using 24 microsatellite markers (SSRs), *in situ* flower manipulation experiments, field survey and observation followed by laboratory identification of insect pollinators, and sensory evaluation using a panel of certified cuppers.

Our results showed strong genetic differentiation between managed (SFC) and unmanaged coffee populations (FC), but we found no significant differences in within-population genetic diversity. The SFC populations, however, were more related to the pool of introduced coffee berry disease (CBD) resistant genotypes than the FC

populations. The widespread planting of coffee seedlings including CBD-resistant cultivars most likely offsets losses of genetic variation attributable to genetic drift and inbreeding. Mixing cultivars with original coffee genotypes resulted in a significant signal of admixture, with a higher mean admixture coefficient in SFC (h_{SFC} = 0.74) than in FC (h_{FC} = 0.30) populations.

C. arabica flowers were visited by a wide range of potential pollinators belonging to sixteen taxonomic groups, comprising 10 insect orders. The most abundant taxonomic groups on coffee flowers were honey bees, butterflies and hoverflies. Taxonomic richness of the flower visiting insects significantly decreased and pollinator community changed with increasing forest management and fragmentation. The relative abundance of honey bees significantly increased with increasing forest management and fragmentation, likely resulting from the introduction of bee hives in the most intensively managed forests.

Our first formal mating system analysis of *C. arabica* in its native range yielded an overall multilocus outcrossing rate of as high as 76% which contrast the wide-held notion that *C. arabica* is a selfing species. A single father could be assigned for 78% of the progenies in highly managed compared to 57% in unmanaged coffee populations, indicating reduced long distance pollen dispersal in managed forests. Furthermore, the fraction of selfed progenies was significantly higher in managed (23%) compared to unmanaged (10%) coffee forests. Finally, neither SFC nor FC populations showed fine scale spatial genetic structure, suggesting high seed dispersal in FC and intense berry harvesting and coffee planting in the SFC.

Contrary to our expectation, coffee flowers received higher pollinator visits in the SFC compared to FC sites. These higher visitation rates in the SFC systems did not result in higher fruit set, however. Fruit set was significantly higher in open pollinated flowers compared to bagged flowers. Coffee forest management did neither affect outcross- nor self pollen limitation, which both appeared to be very low in *C. arabica*. We showed that pollinators play an important role for enhanced productivity of *C. arabica* in its native range.

Highly managed forest fragments (SFC) showed lower scores in nearly all organoleptic quality attributes evaluated than unmanaged forests (FC). The organoleptic quality attributes were not significantly influenced by genotypes and soil physico-chemical properties in the traditional forest coffee production systems of SW Ethiopia. Coffee samples from FC received invariably high quality scores and qualify as speciality coffee.

Our results imply that *in situ* conservation of the wild gene pool of *C. arabica* must focus on limiting intensification of coffee forest management, as intensification threatens the genetic integrity and cup quality of the wild population by exposing them to cultivars, and causes mating pattern alteration and reduced abundance and diversity of pollinators.

SAMENVATTING

Coffea arabica L., beter gekend als wilde koffie, arabica koffie of hooglandkoffie, is de belangrijkste commerciële koffiesoort. Het is van nature een struik uit de onderetage van het Ethiopische regenwoud en de vochtige bossen in het zuidwesten van Ethiopië zijn het centrum van herkomst en het reservoir van genetische diversiteit van de soort. Deze bossen zijn helaas sterk versnipperd, verstoord en gedegradeerd als gevolg van ontbossing voor landbouw, onduurzame houtoogst en bosbeheer in functie van de koffieteelt. In de traditionele koffieteelt in Ethiopië wordt onderscheid gemaakt tussen zogenaamde 'boskoffie', waarin boeren koffie oogsten van wilde struiken in weinig verstoord bos, en 'semi-boskoffie', waarin boeren bomen en struiken kappen om de koffiestruiken meer licht en minder concurrentie te bezorgen waardoor de oogst verhoogt. Er worden ook koffiestruiken aangeplant, en dat kunnen wilde struiken zijn die elders verzameld werden of lokaal gekweekte cultivars die een verhoogde resistentie hebben tegen de koffiebesziekte. In dit eindwerk wordt nagegaan wat de impact is van deze vorm van bosbeheer op de genetische integriteit en diversiteit van de wilde koffie, op de bestuivingsprocessen van koffie en op de kwaliteit van de koffie.

In een eerste luik werd de genetische diversiteit bepaald van koffiepopulaties in sterk en minder sterk beheerde bossen en werd nagegaan of de genetische integriteit van de wilde koffie bedreigd wordt door de introductie van lokaal verbeterde cultivars. Vervolgens werd nagegaan of er aantoonbare effecten bestaan van bosbeheer op de abundantie en diversiteit van mogelijke bestuivers van wilde koffie, op de bestuivingspatronen van wilde koffie, en op vruchtzetting van wilde koffie. Tenslotte werd onderzocht of het bosbeheer een invloed heeft op de kwaliteit van het eindproduct, namelijk de smaak van de koffie. We bepaalden de genetische vingerafdruk van een groot aantal koffieplanten uit beheerd en onbeheerd bos en van alle lokaal ontwikkelde cultivars; we maakten gebruik van experimenten waarbij koffiebloemen op het terrein werden onderworpen aan experimentele behandelingen; we observeerden en identificeerden mogelijke bestuivers van koffie in verschillende bossen; en we lieten een professioneel smaakpanel de kwaliteit van een reeks koffiestalen uit beheerd en onbeheerd bos beoordelen.

De DNA vingerafdrukken wezen erop dat koffiestruiken uit het onbeheerd ‘boskoffie’ systeem en intensief beheerd ‘semi-boskoffie’ systeem genetisch sterk van elkaar verschillen, maar we vonden geen verschil in de genetische diversiteit tussen beide systemen. We vonden wel een sterke overeenkomst tussen de cultivars en de struiken in het semi-boskoffie systeem. De verliezen aan genetische diversiteit die te verwachten waren in de intensief beheerde systemen werden waarschijnlijk gecompenseerd door het aanplanten van koffiestruiken en cultivars. Dit zorgt voor een ‘aanrijking’ van de genenpool, maar de DNA vingerafdrukken toonden duidelijk aan dat het hier dan vooral ging om een bijmenging van cultivar-genen in de wilde genenpool, wat niet wenselijk is.

De bloemen van wilde koffie werden bezocht door een groot aantal mogelijke bestuivers uit zestien taxonomische groepen verdeeld over tien insectenorden. De meest talrijke bezoekers waren honingbijen, vlinders en zweefvliegen. De gemeenschap mogelijke bestuivers in beheerd bos was minder divers en dus ook verschillend van die in onbeheerd bos. In het beheerd bos was het aandeel half-wilde en wilde honingbijen onder de mogelijke bestuivers beduidend hoger. Dit heeft waarschijnlijk te maken met de aanwezigheid van traditionele bijenkorven in de boomkruinen van de beheerde bossen.

De bestuivingsstudie was de eerste voor wilde koffie binnen het natuurlijk verspreidingsgebied van de soort en hieruit bleek dat wilde koffie wel zelfbestoven kan worden maar voornamelijk gebruik maakt van kruisbestuiving (76%). Het aandeel nakomelingen van één zelfde vaderplant en het aandeel nakomelingen voortgebracht uit zelfbestuiving was hoger in beheerde bossen, wat duidt op minder efficiënte en minder verre verbreiding van pollen in deze bossen. In geen van beide bostypen werd een fijnschalige genetische structuur waargenomen. In de niet beheerde bossen wijst dit waarschijnlijk op een efficiënte verbreiding van zaden over grote afstanden, terwijl dit in de beheerde bossen voornamelijk te maken zal hebben met menselijke factoren, zoals het planten van zaailingen die elders verzameld werden en het oogsten van de bessen.

Koffiebloemen werden frequenter bezocht door mogelijke bestuivers in sterk beheerde bossen, maar dit leidde niet consequent tot hogere zaadzetting. Zaadzetting was lager in experimenteel afgesloten bloemen. Pollenlimitatie was zeer laag en werd niet beïnvloed door het bosbeheer. Aangezien zaadzetting het hoogst was in vrij toegankelijke bloemen, blijkt uit deze experimenten dat bestuivers cruciaal zijn voor hoge productiviteit in Ethiopische koffieteeltsystemen.

Koffiestalen uit sterk beheerde bossen kregen beduidend lagere scores voor zo goed als alle smaak- en kwaliteitsvariabelen. Niet zozeer de fysische en chemische bodemvariabelen, maar wel de verwantschap met lokale cultivars bleek sterk gerelateerd te zijn aan deze lage scores. Koffiestalen uit het grote, minst verstoorde bos kregen onveranderlijk hoge kwaliteitsbeoordelingen waardoor deze gerangschikt konden worden als “specialty” koffie, een uitmuntend kwaliteitslabel volgens de standaard van de Specialty Coffee Association of America.

Onze resultaten wijzen erop dat de in-situ conservering van wilde koffie in de eerste plaats moet uitgaan van het voorkomen van verdere intensificatie van koffieteelt in de laatste grote onverstoorde stukken bos. Het is namelijk de verstoring van het bos die samengaat met de intensificatie van de koffieteelt die zorgt voor vervuiling van de wilde genenpool met cultivargenen, verandering van bestuiversgemeenschappen, verstoring van het bestuivings- en vruchtzettingsproces, en uiteindelijk kwaliteitsverlies van de wilde arabica koffie.

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LIST OF ABBREVIATIONS

A	Autofertility
a.s.l	Above see level
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
CBD	Coffee Berry Disease
CWR	Crop Wild Relative
D	Nei's genetic distance
dbh	Diameter at breast height
FAO	Food and Agriculture Organization of the United Nations
FC	Forest Coffee
G_{st}	Nei's Population Differentiation
H'	Shannon-Wiener Diversity Index
H'c	Shannon-Wiener Diversity Index Corrected for Sample Size
HWE	Hardy Weinberg Equilibrium
ICO	International Coffee Organization
LOD	Likelihood ratio
MLGs	Multilocus Genotypes
MRPP	Multi-Response Permutation Procedure
MV	Molecular Variance
NFPA	National Forest Priority Area

NMS	Non-Metric Multi-dimensional Scaling
PC	Principal Component
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PLs	Self Pollen Limitation
PLx	Outcross Pollen Limitation
RA	Reproductive Assurance
SCAA	Specialty Coffee Association of America
SFC	Semi-forest Coffee
SGS	Spatial Genetic Structure
SSR	Simple Sequence Repeat
SSWP	Within Population Sum of Squares

CHAPTER 1:

GENERAL INTRODUCTION

1.1 Crop Wild Relatives and their importance

Crop wild relatives (CWRs) are wild plant taxa that are phylogenetically closely related to crop species (- including the wild populations of the crop species itself) of direct socioeconomic importance, and which possess desirable traits that can be bred into existing crops (Maxted *et al.* 2006; Meilleur and Hodgkin 2004). CWRs are important components of plant genetic resources for food production and agriculture in general worldwide, and are expected to contribute greatly to future food security. Between 1986 and 2006, 60 CWRs have contributed more than 100 beneficial traits, mainly related to disease resistance and abiotic stress tolerance, to 13 major crops (Hajjar and Hodgkin 2007). Furthermore, it has been estimated that 30% of the increase in crop yields since 1945 has been achieved through crossing crop species with their CWRs, representing a worldwide value of US$115 billion per year (Pimentel *et al*. 1997). The importance of CWRs can be expected to increase in the future as plant breeders attempt to address the threats posed by the combination of global environmental change and a higher demand for food (Foley *et al*. 2011). At the same time, recent advances in molecular and breeding techniques increasingly allow efficient introduction of genes from more remote relatives into crop species (Hajjar and Hodgkin 2007; Shapter *et al.* 2009; Varshney *et al*. 2009).

1.2 Conservation of crop wild relatives

CWRs are a finite resource that is being eroded or lost owing to irresponsible human practices (Maxted *et al*. 2007). Globally, CWRs are subjected to a range of increasing threats including urbanization, deforestation, habitat fragmentation, intensification of farming practices and climate changes (Maxted and Kell 2009). Although conservation efforts of CWRs using both *in situ* (on site or in the natural habitat) approaches and *ex-situ* (off site in gene banks and botanical gardens) approaches date back to the beginning of 20 century, the progress made so far is very modest, and the conservation of many CWRs is simply neglected as most of them grow outside protected areas (Maxted *et al*. 2007, Honnay et al. 2012). The conservation of CWRs, and of their extant genetic diversity is, however, of major importance, and an often undervalued challenge for conservation biologists (Meilleur and Hodgkin 2004; Honnay *et al*. 2012). This PhD

study has the focus on one of the most economically important CWRs: The Ethiopian wild populations of worldwide cultivated Arabica coffee (*Coffea arabica*).

1. 3 The coffee genus

Coffees belong to the large angiosperm family *Rubiaceae* and are classified into two genera: *Coffea* and *Psilanthus*. Charrier and Berthaud (1985) subdivided the genus *Coffea* into two subgenera: *Coffea* (*Eucoffea*) and *Mascarocoffea*. These authors also indicated that caffeine-containing coffee shrubs belong to the subgenus *Coffea*. According to the pre-phylogenetic circumscription, *Coffea* species were believed to originate from tropical forests of Africa, Madagascar and islands of the Indian Ocean (Mascaren islands), whereas coffee species described under the genus *Psilanthus* occur in Asia and tropical Africa (Davis *et al.* 2006; 2010). However, based on recent evolutionary work using molecular and morphological markers, Davis *et al.* (2011) subsumed the genus *Psilanthus Hook. f.* into *Coffea* and increased the total number of coffee species currently described to 124 species (Davis *et al.* 2006; 2007; 2010; 2011).

1. 4 Economically important coffee species in Ethiopia and beyond

Coffee is the world's most important legally traded agricultural commodity (Vega 2008). It is considered the most important tropical commodity that contributes to nearly half of the total net exports of tropical products (Hallam 2003). Currently, about 80 tropical countries produce and export coffee, generating a significant amount of income (Tesfaye *et al.* 2013). Being cultivated on ca. 11.5 million ha of land globally, the coffee industry directly involves ca. 25 million farmers and 125 million people (Waller *et al.* 2007). Commercial coffee production involves mainly two species namely *C. arabica* L (Arabica or highland coffee) and *C. canephora* Pierre ex Froehner (robusta or lowland coffee) (Anthony *et al.* 2001; 2002). *C. arabica* accounts for two-third of the world coffee production owing to its better cup quality, low bitterness and good flavor whereas the remaining production volume comes from *C. canephora*, which is mainly known for its high caffeine content (Labouise *et al.* 2008).

C. arabica is the only coffee species grown in Ethiopia, and it plays a significant role in Ethiopian economy, contributing over 35 % of the total export value; 4 to 5% to

the national Gross Domestic Product and generating 20% of government revenue (Petit 2007). Coffee also plays a central role as source of income for over one million coffee growing households, and over 15 million people derive their livelihood directly or indirectly from this crop along the value chain (Petit 2007; Labouisse *et al.* 2008). National production levels are estimated to be 396,000 tons per annum (ICO 2013), of which close to 50% are domestically consumed. Ethiopia is the third largest Arabica coffee producer in the world following Brazil and Colombia in that order (ICO 2013).

1. 5 Morphological and phenological descriptions of *C. arabica*

Highland coffee or *C. arabica* is a short, woody perennial shrub that grows to 3-12m if not pruned (Witngens 2012). *C. arabica* shrubs require 3-4 years, starting from time of seed germination, to flower and fruit bearing (Witngens 2012). It is mostly grown in the tropical and subtropical regions of the world (Davis *et al.* 2006). It has an extensive root system and most roots that are responsible for nutrients and water absorption are concentrated in the top 0- 30cm of the soil layer, although some roots can even extend down to 3m depth (Vieira 2008). As almost all species of the *Coffea* genus, it has evergreen opposite, petiolate and glabrous leaves (Witngens 2012). Highland coffee has a single main trunk that has a dimorphic branching habit in which vertical (orthotropic) shoots extend from the main trunk and form horizontal (plagiotropic) ramifications which bear flowers and fruits in clusters (Davis *et al.* 2006; Witengen 2012). Flowers are white and supported by a short pedicel with a rudimentary five-sepal calyx. The salver-shaped corolla is 4-9 lobed (Fig.1.1). The anthers are relatively short and inserted at the throat of the corolla tube by a short filament (Free 1993; Witengen 2012).

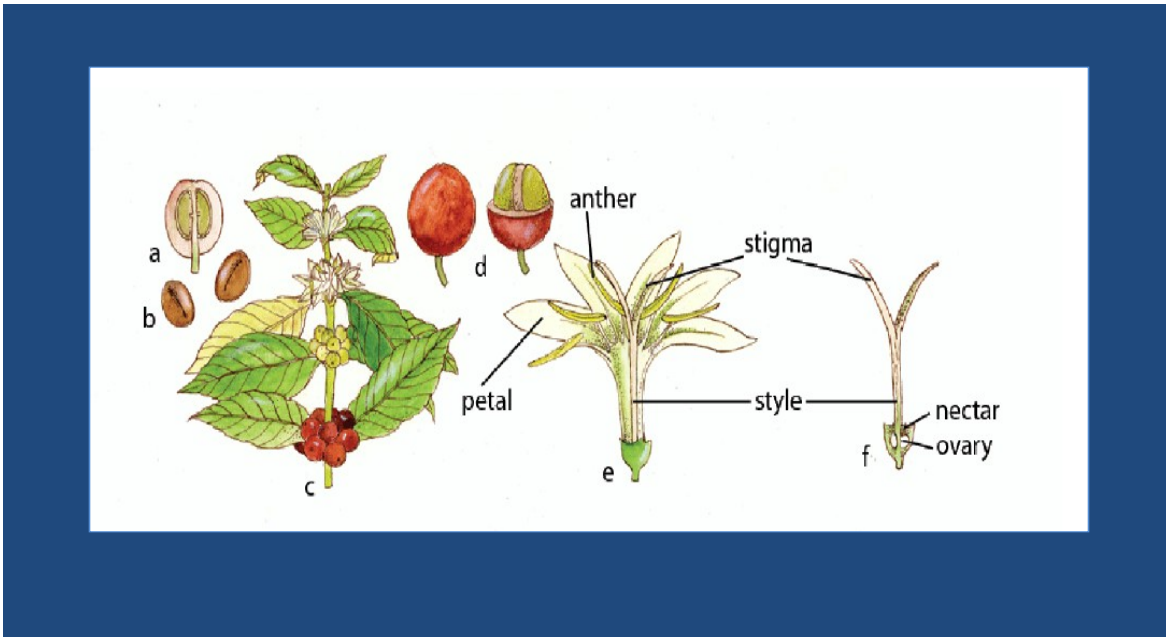


Figure1.1 Longitudinal view of berry (a), beans (b), flowering and fruiting branch (c), berry (d), longitudinal view of flower (e) and longitudinal view of female flower parts (f) of *Coffea arabica:* adapted from Free (1993).

Flowering in highland coffee is induced by rain showers, with a short annual period of synchronous flowering usually between January and April. Fruit maturity takes 7 to 9 months, depending on the location. *C. arabica* fruits are ellipsoid, obovate “drupes” (fleshy fruits that have a hard nut) and normally develop with two ovules that result in two beans within a fruit (De Castro and Morraccini 2006). However, abnormal fruit development resulting in abnormal and misshaped beans is not uncommon. An example of such an abnormally formed coffee bean is a “pea berry”. Pea berries occur when only one ovule matures and one is aborted during fruit development, resulting in one seed (Free 1993; Witngens 2012). Although pea berries are commercially generally undesirable due to their shape (deformed or misshapen), there is a niche market for them (Ricketts *et al.* 2004).

1. 6 Origin and diversity of *Coffea arabica*

Wild Arabica coffee has its center of origin and diversity in the highlands of southwestern and southeastern Ethiopia (Sylvain 1955), where it occurs as an understory shrub in the moist evergreen Afromontane forests, and where it has been grown as an understory shrub in managed forests for centuries (Friis 1992). The exact date of *C. arabica*'s first departure from its center of origin, Ethiopia to other parts of the world is not precisely documented. However, what is known is that the coffee plant first made its way from Ethiopia to Yemen (Wellman 1961; Vega 2008). *C. arabica* spread to different countries of the world from Yemen via different routes. Coffee seeds moved from Yemen to the Dutch colony of "Java" in 1960 through the Dutch East India company (Steiger *et al.* 2002). Ten years later, coffee plants were transferred from Java to the Amsterdam Botanical Garden and these plants gave rise to the botanical variety of *C. arabica* called "*Typica"* (Wellman 1961). By 1713, a single plant made its way from the Amsterdam botanical garden to France. Although two coffee plants started their way from France in 1720, only one plant reached the French colony of Martinique (Vega 2008). Following this introduction, it took few years to spread throughout the Caribbean Islands. Nearly at the same time, the Dutch introduced coffee from the Amsterdam Botanical garden to the South-American colony of Suriname, and the progeny of these plants were first introduced to French Guiana, and then to Brazil in 1727 (Vega 2008). In 1718 the French transported new plants from Yemen to the Bourbon Islands, now called Reunion. These introduced coffee plants produced small beans and gave rise to the other botanical variety of *C. arabica* known as "*Bourbon*". Finally, Brazilian coffee cultivars travelled back to Africa in 1893, ending the transcontinental journey of *C. arabica* (Fig.1.2).

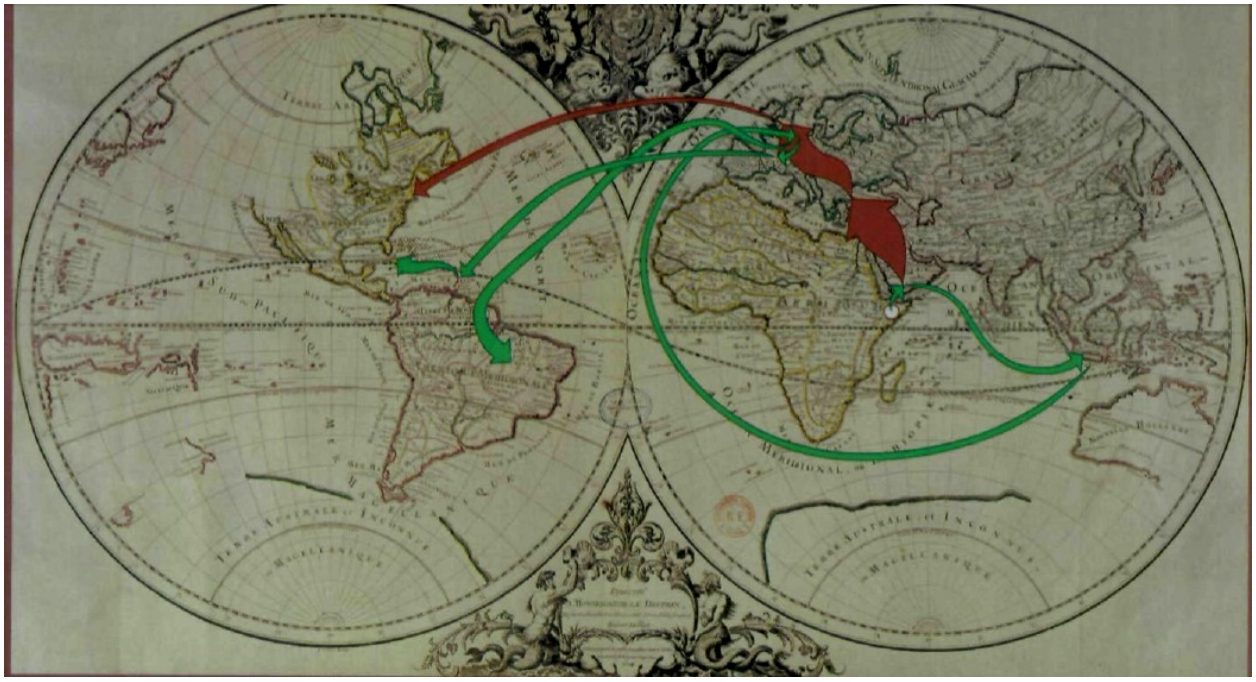


Figure 1.2 Transcontinental journey of *C. arabica* from its center of origin, Ethiopia to the rest of the world. Green arrows show the travel to countries where *C. arabica* was cultivated; red arrows show the spread of coffee as a beverage (Vega 2008).

The wild *C. arabica*, and its cultivated varieties growing worldwide, are naturally autogamous (self-fertile), and they are the only tetraploid taxon (allotetraploid, 2n = 4x = 44) in the Genus *Coffea*. All other coffee species are diploid (2n = 22) and generally self-sterile (Lashermes *et al.* 1999; Davis *et al.* 2006). An allotetraploid origin was already suggested for Arabica coffee by Carvalho (1952) as the species shows a diploid-like meiotic behavior and has its center of genetic diversity outside the distribution area of the diploid species of the genus. Lashermes *et al.* (1999) suggested that *C. arabica* is an amphidiploid formed by hybridization between *C. eugenoides* and *C. canephora,* or ecotypes related to these diploid species. Arabica coffee has a relatively narrow genetic base as compared to its diploid counterparts. Cultivated commercial cultivars of Arabica coffee that are currently under production in the major

producing countries of the world are criticized for having a very narrow genetic base, attributable to their predominantly autogamous nature (Lashermes *et al.* 2000). Furthermore, and as explained above, they represent only a very small proportion of the potential genetic diversity available within the Ethiopian coffee gene pool due to the subsequent genetic bottlenecks that characterized the spread of coffee plants throughout the world (Orozco-Castillo *et al.* 1994, Anthony *et al.* 2002). Studies using morphological and molecular markers (Lashermes *et al.* 1996; Anthony *et al.* 2001; 2002; Denich and Gatzweiler 2006) have indeed suggested high genetic diversity in the wild Arabica coffee populations in their center of origin, SW Ethiopia, and a clear genetic differentiation between wild populations, commercial cultivars and landraces which were obtained from accessions (Denich and Gatzweiler 2006). However, these studies analyzed genetic diversity of *C. arabica* individuals from gene bank material, using less informative DNA markers such as RAPDs. Also the number of populations sampled and analyzed were rather low. A comprehensive assessment of the *in situ* genetic diversity of *C. arabica* in SW Ethiopia using more polymorphic DNA markers such as microsatellites (SSRs) is lacking so far.

1. 7 Coffee production systems in Ethiopia

Although traditional coffee production systems in SW Ethiopia are similar to rustic coffee production systems in some Latin American countries where coffee is grown under a canopy cover of indigenous trees (e.g. Hernandez-Martinez *et al.* 2009), they are different in that the coffee shrubs are endemic to Ethiopian forests and a natural part of the understorey. Over 90% of the coffee production in Ethiopia is produced by smallholder farmers (Labouisse *et al.* 2008). Woldetsadik and Kebede (2000) distinguished four major coffee production systems in Ethiopia, largely based on the magnitude of human interventions and domestication: forest coffee (FC), semi-forest coffee (SFC), garden coffee and plantation coffee production systems (Fig.1.3). Forest coffee production systems, which account for 5% of the total national production (Petit 2007), refer to simple coffee gathering where coffee trees are protected and sometimes tended for convenient picking. The productivity of this production system is very low and has been estimated at c. 15 kg ha^{-1} (Schmitt *et al.* 2009). SFC systems have evolved from forest coffee systems due to anthropogenic activities, and account for 35% of the

total national production. In this case, the farmers practice slashing (of weeds, competing shrubs and thin forest trees), and gap filling with locally obtained coffee seedlings. The average productivity of this system is still low (54 kg ha^{-1}) (Schmitt *et al.* 2009). In garden coffee production system, seedlings are collected from forest coffee or other sources, and they are transplanted closer to farmers' dwellings. Coffee is grown in smallholdings under a few shade trees, usually combined with other crops (such as maize, sorghum and banana) and fruit trees such as avocado and mango. This production system accounts for about 50% of the national production (Petit 2007), with an average productivity of 650 kg ha^{-1} per year. The plantation coffee system, finally, which uses modern management practices to boost productivity and quality, accounts for 10% of the national coffee production. It includes a few large privately and state owned farms as well as many smallholder plantations spread all over the coffee growing regions of the country. Unlike the other systems, the state owned coffee plantation systems apply recommended fertilizers, chemicals for the control of pests and improved and disease resistant cultivars. Except for two coffee leaf rust resistant cultivars introduced from Portugal, over 95% of the cultivars currently used in the plantation coffee production system are locally improved coffee berry disease (CBD) resistant cultivars. Some good yielding landraces are also part of the system, although they are rather the preferred planting materials in the garden coffee production system. "Landraces" are coffee cultivars domesticated and commonly grown by farmers close to their dwellings (Labouisse *et al.* 2008). These landraces often resulted from a complex process of transportation from one region of the country to another, exchanges and selection by farmers and adaptation to environments which are sometimes different from their original habitat (Labouisse *et al.* 2008). The coffee berry disease (CBD) resistant cultivars currently grown in Ethiopia were developed through selection and intraspecific hybridization of *C. arabica* accessions collected from different Afromontane evergreen coffee forests of the country, as an immediate solution for the catastrophic outbreak of the CBD in 1970s (Labouisse *et al.* 2008).

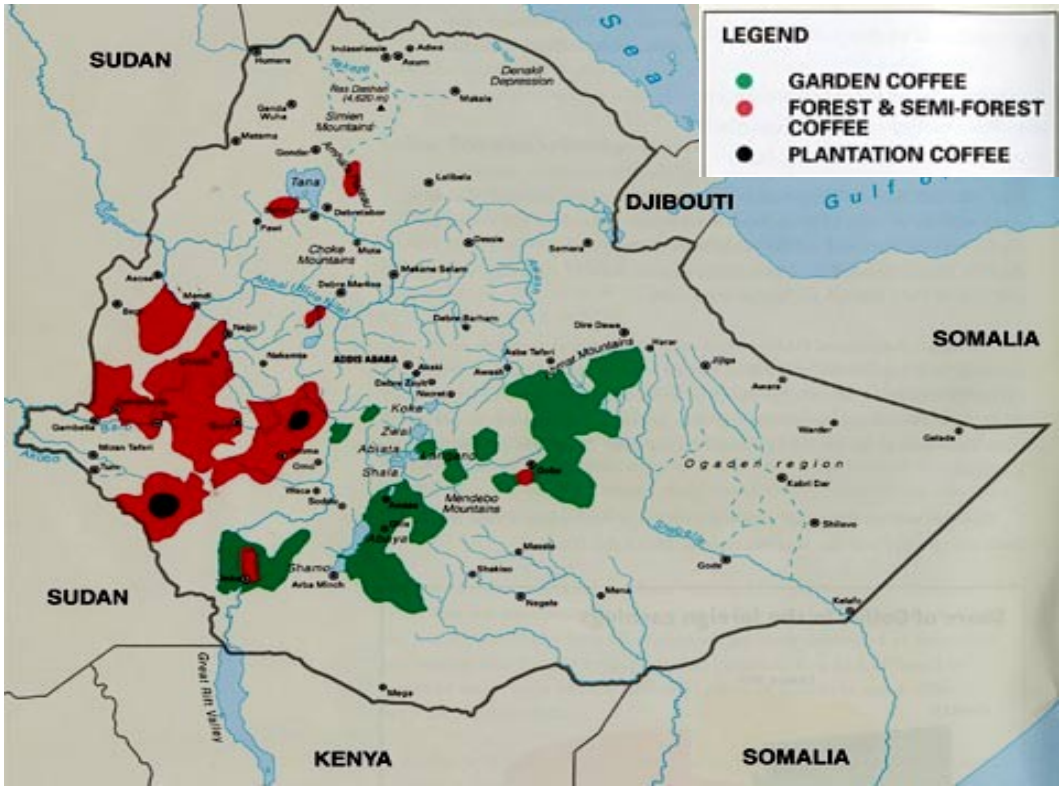


Figure 1.3 Major Arabica coffee growing areas of Ethiopia.
[www.treecrops.org/country/ethiopia.htm]

1. 8 Diseases and insect pests of *Coffea arabica* in Ethiopia

Highland coffee is susceptible to different diseases and insect pests in Ethiopia. Among the documented coffee diseases in the country, coffee berry disease (*Colletotrichum kahawae*), coffee wilt disease (*Gibberella xylarioides*) and coffee leaf rust (*Hemileia vastatrix*) are the most damaging fungal diseases, and their prevalence in all coffee production systems have been documented (Zeru *et al*. 2008). Nevertheless, the prevalence of these diseases varies significantly from location to location and from one production system to another. For instance, coffee berry disease (CBD) is reported to cause yield losses up to100% in some localities, and little to none in some other localities, with the national average crop losses ranging from 25% to 30% (Zeru *et al*.

2008). In addition to the above mentioned major diseases, other diseases of less importance in traditional coffee production systems have been reported. These include damping-off caused by *Fusarium*, *Pythium*, *Rhizoctonia* and *Mucor* spp, Armillaria root rot (*Armillaria mellea* (Vahl ex Fries) Kummer), coffee bean discoloration/rot caused by *Pseudomonas syringae,* Brown eye-spot (*Cercospora coffeicola* Berk. and Cke), Aschochyta leaf blight (*Ascochyta tarda* Stewart), and coffee moulds caused by *Aspergillus spp*, *Penicilium spp* and *Fusarium spp.* Most of these fungi are reported to be endemic to Ethiopia. Unlike the above three important fungal diseases, they are less damaging which most probably emanates from co-evolution between the host and the pathogen over centuries. Nevertheless, the status of some diseases has been dynamically changing in recent years, possibly due to the planting of new coffee varieties, and improved management practices allowing coffee to grow under sub-optimal conditions, resulting into an imbalance in the host-pathogen-environment interactions (Zeru *et al*. 2008).

Compared to plant diseases, insect pests are less damaging to Ethiopian coffee cultivation, and have remained less important as compared to many other coffee producing countries throughout the world. In Ethiopia, over 47 insect species have been reported to attack *C. arabica*. Among the reported insect pests in the country, two insect pests, the Antestia bug (*Antestiopsis intricata*, *A. facetoides*) and the coffee blotch miner (*Leucoptera caffeinia)* are the major ones, inflicting considerable yield and quality losses. Other insect pests such as the coffee berry borer, (*Hypothenemus hampei)*, coffee thrips (*Diarthrothrips coffeae)*, green scale (*Coccus alpines*) and coffee cushion scale (*Stictococcus formicarius*) are considered to be potentially important (Mendesil *et al*. 2008). Observational studies conducted in SW Ethiopia indicated that insect pest damages are more pronounced in intensive coffee production systems (plantations) compared to garden and semi-forest coffee production systems, probably due to changes in cultural practices associated with newly planted cultivars (Mendesil *et al*. 2008). One of the possible reasons for the low occurrence of insect pests in the least managed and more genetically diversified forest coffee populations is the existence of

diverse natural enemies, which keep the insect populations at low levels, and maintains a pest vs. natural enemy balance (reviewed in Mendesil *et al*. 2008).

1. 9 Forest degradation and fragmentation and its biodiversity consequences

An estimated 77% of the earth's ice free land has now been significantly changed by human activities, and that proportion is likely to increase (Rands *et al.* 2010). Of this 77%, close to 40% is being directly used by humans for agriculture and urbanization whereas 37% of it is surrounded by landscapes that are strongly anthropogenically modified (Ellis *et al.* 2010). The continued growths of both human populations worldwide and of the per capita food consumption have exacerbated these pressures on the planet (Rands *et al.* 2010). Anthropogenic activities have also resulted in the degradation and fragmentation of natural habitats (e.g Aguilar *et al.* 2008; Steffan-Dewenter and Westphal 2008), overexploitation of species (Butchart *et al.* 2010), increased abundance of invasive alien species (Bjerknes *et al.* 2007; Traveset and Richardson 2006), and climate change (Memmott *et al.* 2007). These anthropogenic activities have numerous consequences for the biological resources of the planet and for ecosystem service provisioning (Sala *et al.* 2000; Fahrig 2003) because of their detrimental effects on genetic and ecological processes (e.g. inducing genetic bottlenecks, alteration of biotic interactions and biological invasions) and on ecosystem functions (Vanbergen *et al.* 2013). Such habitat alterations not only cause local extinctions but also may force the remaining individuals to live in small and spatially isolated habitat fragments, often of degraded quality. Habitat fragmentation causes genetic erosion as a consequence of genetic drift, inbreeding and reduced gene flow (through pollen or seed) within and among small and isolated populations (Honnay and Jacquemyn 2007). Furthermore, abundance and diversity of pollinators becomes reduced in small and isolated natural habitats, thereby jeopardizing pollination efficiency, reproductive output, and ultimately plant species richness (Aguilar *et al.* 2006; Potts *et al.* 2010). Habitat fragmentation, particularly forest fragmentation, is more pronounced in tropical forest landscapes (Foley *et al.* 2005). Studies showed that many continuous forests in tropical landscapes have been converted or are in the midst of being converted to agriculture and other land use systems (Mortn *et al.* 2006; Brink and Evan 2009; Hylander *et al.* 2013). Tropical forests have also been dramatically changed by human disturbance and forest

management, particularly in densely populated areas (Lows *et al.* 2005; Aerts *et al.* 2011). In such highly populated areas, wood harvesting through removal of canopy trees is very common and may have important consequences for forest microclimate, for pollinator abundance, diversity and foraging behavior, possibly affecting plant reproduction and hence plant species diversity (Eckert *et al.* 2010).

1.10 Forest resources of Ethiopia

Located in the horn of Africa, Ethiopia is one of the top 25 biodiversity rich countries of the world (Teketay 2001), with varying landscapes ranging from high and rugged mountains, flat-topped plateaus, deep gorges, incised rivers, valleys, and rolling plains (Teketay 2001; Gebre-Egziabher 1991). The occurrence of these variable landscapes has contributed to the formation of diverse ecosystems featured by great species diversity (e.g. Pankhurst 1995). Also the forest areas of Ethiopia are well recognized for their high biodiversity, which is associated with great economic and ecological significance (Hein and Gatzweiler *et al.* 2006). About a century ago, close to 40% of the total area of the country was covered by high forests (Bekele and Berhanu 2001), but this declined at an alarming rate to a value below 3% by the late 1980s (Rogers 1992; EFAP 1994). The concerted effects of conversion of forests to arable land, over-exploitation/harvesting, overgrazing, fire and government settlement programs, all exacerbated by an ever increasing human population pressure, are the main causes of this reduction of forest cover (Friis 1992; Gole 2003; Getahun *et al.* 2013). Currently, moist evergreen Afromontane forests are the major remnant forests in the country (Gole 2003), and they are mainly confined to fragmented patches in southwestern regions: Oromiya, Gambela and Southern Nations, Nationalities and Peoples' Region (SNNPR), or the former administrative regions Kefa, Illubabor and Wellega (Senbeta 2006). These forests occur at an elevation between 1500m and 2600m, with mean annual temperatures and rainfall ranging between 15-20^{o}C and 700 to 2500mm, respectively (Friis 1992).

1.11 Threats to wild Arabica coffee in Ethiopia

The Afromontane evergreen rain forests of Ethiopia constitute the native habitat for wild *C. arabica* (Senbeta and Denich 2006; Gole *et al.* 2008; Schmitt *et al.* 2013). Wild

populations of Arabica coffee in these rainforests are genetically diverse and they may contribute to the genetic diversification of Arabica coffee production worldwide, as they likely possess desirable traits that can be used to improve the cultivated varieties of *C. arabica*, or increase their disease resistance. Nevertheless, like other forests of the world, the Afromontane forests of Ethiopia that harbor the wild Arabica coffee gene pool, have been under continuous threat due to habitat destruction (Gole *et al.* 2003; Aga *et al.* 2005; Senbeta and Denich 2006), forest fragmentation and intensification of coffee management practices (Schmitt *et al.* 2009; Aerts *et al.* 2011). The low yields that are typical of forest-based coffee production systems (Schmitt *et al.* 2009) have often lead to intensification of the traditional and low intensity forest and semi-forest coffee systems, with local farmers uprooting wild shrubs and replacing them with high yielding coffee cultivars or other cash crops like 'Chat', *Catha edulis* (Mekuria *et al.* 2004). Such anthropogenic disturbances are likely affecting the genetic diversity, mating patterns, gene flow, pollinator abundance and diversity, and microclimate, thereby influencing yield and long term viability of the residing coffee populations.

1. 12 Effects of Afromontane forest fragmentation and management on coffee

1.12.1 Genetic diversity of wild coffee

Anthropogenic forest fragmentation and deforestation, as well as current forest management are possibly threatening the genetic diversity of the Arabica coffee populations (Schmitt *et al.* 2009; Aerts *et al.* 2011). First, habitat fragmentation may directly lead to the loss of alleles through genetic drift, particularly in isolated habitats where alleles lost through drift cannot be replenished through gene flow (by pollen and seed). Second, intensification of forest coffee (FC) system degrades forest habitat and disrupts coffee population structure. Third, introduction of remote landraces and improved high yielding and disease resistant cultivars of Arabica coffee into fragments threatens natural genetic diversity of wild coffee due to the potential effects of introgression and outbreeding. Introgression is "the permanent incorporation of genes from one set of differentiated populations (species, subspecies, races and so on) into another population" (Rhymer and Simberloff 1996). Contamination of wild populations with domesticated alleles is an important consideration when identifying populations to

conserve *in situ* (Green *et al.* 2008). Generally, reduced gene flow between subpopulation, loss of alleles due to genetic drift and hybridization (genetic pollution) are expected to cause genetic erosion of the wild coffee gene pool. Although outbreeding is advantageous for enhancing genetic variability in population, it has certain drawbacks particularly when spatially separated and genetically differentiated gene pools are mixed. One of the noticeable disadvantages of outbreeding is outbreeding depression, which is the reduction in offspring performance (i.e. fitness) relative to the parents (Lynch 1991). This fitness decline may arise due to the disruption of adaptation to local conditions (through mixing of gene pools that are adapted to the local environment, resulting in offspring that is adapted to neither of the parental environments), or the disruption of co-adapted gene complexes that have evolved in either population, or a combination of both mechanisms (Lynch 1991).

Empirical studies of the impacts of anthropogenic forest disturbance and management on the genetic diversity of Ethiopian wild coffee populations, and on the degree of introgression of alleles from locally improved disease resistant cultivars are scarce or absent. Although few prior studies (Anthony *et al.* 2001; 2002; Aga *et al.* 2003; 2005; Tesfaye 2006; Silvestrini *et al.* 2007) reported high genetic diversity of *C. arabica* in Ethiopia, in-depth insight on the level and structuring of genetic diversity of wild coffee using DNA based-molecular markers such as SSR is still needed.

1.12.2 Mating patterns

Besides their effect on genetic diversity, fragmentation and destruction of natural forests for coffee cultivation may alter mating patterns in plant populations (Eckert *et al.* 2010), and more specifically, often decrease opportunities for outcross pollination (Aguilar *et al.* 2006). Estimating outcrossing and selfing rates at different spatial scales is pertinent to understand the reproductive biology and landscape genetics of the plant species under question (Eckert *et al.* 2010). Outcrossing rates have been shown to be low in disturbed habitats (Obayashi *et al.* 2002; Eckert *et al.* 2010), attributable to a reduction in the amount of outcrossed pollen deposited on stigmas (reviewed in Eckert *et al.* 2010). Such reduction in outcross pollen delivery could be due to lower pollinator abundance, smaller and sparser plant populations that attract fewer pollinators, or the interaction of

both. Several biotic and abiotic agents are speculated to affect gene flow in wild coffee in Ethiopia. Man could be regarded as one of the agents of gene flow via seedling exchange of preferred wild populations, thinning and filling up gaps in the SFC with self sown seedlings collected from other forest fragments (Gole *et al.* 2003; Aga 2005; Senbeta and Denich 2006), or high yielding and disease resistant cultivars from governmental institutions. Comparison of matting patterns differences between FC and SFC system as a function of forest fragmentation and forest management for coffee cultivation is therefore required.

1.12.3 Abundance and diversity of pollinators

It has been well documented that anthropogenic disturbance may result in loss of pollinator diversity and alteration of the plant-pollinator interactions (Potts *et al.* 2010; Garibaldi *et al.* 2011). Plant-pollinator interactions can be altered by habitat fragmentation (Aguilar *et al.* 2006; Cane *et al.* 2006; Brosi *et al.* 2008; Steffan-Dewenter and Wastphal 2008), invasion by non-indigenous competitors, pollinators and herbivores (Traveset and Richardson 2006; Bjerknes *et al.* 2007), and climate change (Hegland *et al.* 2009; Memmot *et al.* 2007). According to Bawa (1990), 89-99% of all flowering plant species of tropical rainforests are pollinated by animals. *C. arabica* is known to benefit from insect pollination (Klein *et al.* 2003), and fruit set increased in agroforestry sites adjacent to natural habitat (Klein *et al.* 2003; Ricketts 2004). Arthropod and pollinator diversity was reported to decline with intensification of coffee management (Klein *et al.* 2003). The effect of traditional coffee management intensity that involves rigorous thinning of canopy trees, removal of undergrowth shrubs in SFC system on the abundance, diversity and visitation rate of *C. arabica* pollinating insects is not documented, however.

1.12.4 Pollen limitation and reproductive assurance

Studies showed that pollination failure and increased selfing and inbreeding are important threats to reproduction within human altered habitats (e.g. Wilcock and Neiland 2002). Anthropogenic activities such as forest fragmentation and forest management may negatively affect pollinator diversity, and may also alter plant-pollinator interactions (Eckert *et al.* 2010; Winfree *et al.* 2011). Scarcity of mates and/or

pollinators can lead to pollen limitation and a decrease in reproductive performance of plant populations within the fragmented habitat (Eckert *et al.* 2010). Pollen limitation (PLx) is defined as a reduction in fruit and seed production caused by a scarce pollen receipt (Knight *et al.* 2005; 2006). This can be due to a reduction in the quantity and/or quality of pollen deposited on the stigma (Knight *et al.* 2005; Aizen and Harder 2007), which possibly results in lower ovule fertilization and seed production or less vigorous offspring (Eckert *et al*. 2010). Therefore, insufficient pollination is the most prominent cause of reproductive impairment in fragmented habitats (reviewed in Aguilar *et al.* 2006).

Plants may also undergo evolutionary changes in response to changing conditions in their habitat, and such conversions have happened many times in many plant species (Eckert *et al.* 2010; Dart and Eckert 2013). For instance, chronic outcross pollen limitation in disturbed habitats (Morgan *et al.* 2005; Knight *et al.* 2006) selects for selfing as a means of reproductive assurance. Reproductive assurance is defined as an increase in seed production afforded by self pollination when scarcity of pollinators or mates limits outcross pollination (Eckert *et al.* 2010). The response of different species to outcross pollen limitation is different, however. Self-compatible species generally show more diverse responses to outcross pollen limitation than self-incompatible ones. Self fertilization through floral mechanisms (autonomous autogamy) provides reproductive assurance by compensating for a shortage of outcross pollen (Eckert *et al.* 2010). However, the extent to which autonomous selfing increases seed production depends on the survival of self versus outcrossed fertilized embryo to seed maturation, as self-fertilized embryos might not survive to the seed stage (Husband and Schemske 1996).

1.12.5 Cup quality of wild Arabica coffee

The unique nature of Ethiopian coffee among others, is that it is being cultivated under different types of shade tree canopy (Gole 2003; Senbeta and Denich 2006). Studies have documented a positive relationship between coffee cup quality and shade management (e.g. Bosselmann *et al.* 2009). The overall beverage quality of *C. arabica* depends on the type of coffee, growing conditions (soil conditions, climatic conditions),

post harvest processing and handling methods (Bertrand *et al.* 2006). Forest fragmentation and intensive management of coffee forests may alter the microclimates in fragmented forests, altering plant growth and development (Schmitt *et al.* 2009; Aerts *et al.* 2011). In SFC systems, the typical coffee management aiming at increasing coffee shrub productivity through decreasing canopy closure, often results in a simplified forest structure, characterized by fewer and thinner stems, lower canopy height and reduced crown closure (see above, Senbeta and Denich 2006; Aerts *et al.* 2011; Hundera *et al.* 2013a). Although very few studies that assessed the quality profile of coffee beans, little is known regarding the influence of intensive forest management typical of SFC system on the cup quality of Arabica coffee in its native range.

1. 13 Aims and thesis outline

The general aim of this study was to quantify the effects of Ethiopian Afromontane coffee forest fragmentation and forest management on wild coffee genetic diversity, reproduction, and cup quality.

Our specific objectives are to:

1. Assess the extent of within and among population genetic diversity of wild Arabica coffee in SW Ethiopian rainforests along a management intensity gradient;
2. Assess the introgression risk of introducing distant landraces and improved cultivars in wild Arabica coffee populations;
3. Quantify the abundance and diversity of pollinators of wild coffee along a gradient of increasing fragmentation and management of Afromontane coffee forests of SW Ethiopia;
4. Quantify the effects of increasing forest management intensity on mating patterns in wild *C. arabica* populations;
5. Investigate the effects of forest fragmentation and forest management intensity on pollen limitation and fruit set in wild *C. arabica* populations;
6. Quantify the effects of forest management intensity, genotypes and soil properties on the organoleptic quality of *C. arabica*

Study sites and sampling design

Our observations and experiments were performed in (i) randomly selected forest fragments with SFC management in the Manna district; and (ii) the nearest forest with FC management, the Gera sector of the Beleta-Gera NFPA in the Gera district. We are aware that differences between the two management systems are potentially confounded by geographical differences between study sites. There were, however, no FC fragments in the cluster of SFC forest fragments, and no isolated SFC fragments in the FC sector. We account for potential differences between study sites through including site characteristics such as soil variables in our models. Hereafter we provide a concise account of the three main study landscapes.

The Garuke site is located at ca. 15km northwest of Jimma, near the rural village 'Garuke' (Fig.1.5A). The mosaic landscape of the Garuke site is dominated by crop production (mainly maize and teff), homegardens, grazing lands and many small forest fragments, either managed for coffee cultivation, or consisting of exotic *Eucalyptus* plantations (Aerts *et al.* 2011). These forest fragments are small (ca. 0.5-9ha), often spatially separated by hundreds of meters, and they have been intensively managed for years. Forest management for coffee cultivation in this landscape (SFC system) involves intensive thinning of the shade tree canopy, removal of shrubs and climax trees, repeated slashing of weeds, and introduction of domesticated coffee landraces and CBD resistant coffee cultivars (Aerts *et al.* 2011). Thinning of the canopy has resulted in the disappearance of almost all late-successional tree species. The remaining canopy includes *Albizia gummifera, A. schimperiana, Croton macrostachyus* and *Millettia ferruginea.* As the different fragments are owned by different farmers, the type of management and management intensity in these fragments are expected to vary from owner to owner. To account for such management intensity variation among farmers, we sampled more fragments as compared to the two study landscapes (Fetche and Gera).

The Fetche site is located at about 10km west-northwest from Jimma town. The Fetche forest is highly managed, but much larger (100ha) than the Garuke forest fragments. The forest was sampled through randomly establishing forest blocks (Fig.1.5B). Forest trees present are comparable with the Garuke fragments.

The Gera site is part of the Belete-Gera National Forest Priority Area and located at 70 km in west of Jimma town. Within Gera forest complex we sampled two large continuous undisturbed forests blocks: Qacho and Afalo (Fig. 1.6A and B). Dominant tree species in this forest include *Syzygium guineense, Prunus africana, Olea welwitschii, Schefflera abyssinica* and *Ilex mitis.* A detailed overview of the study sites and of the number of plots sampled for each chapter of this manuscript is provided in Appendix Table 1.1.

A

B

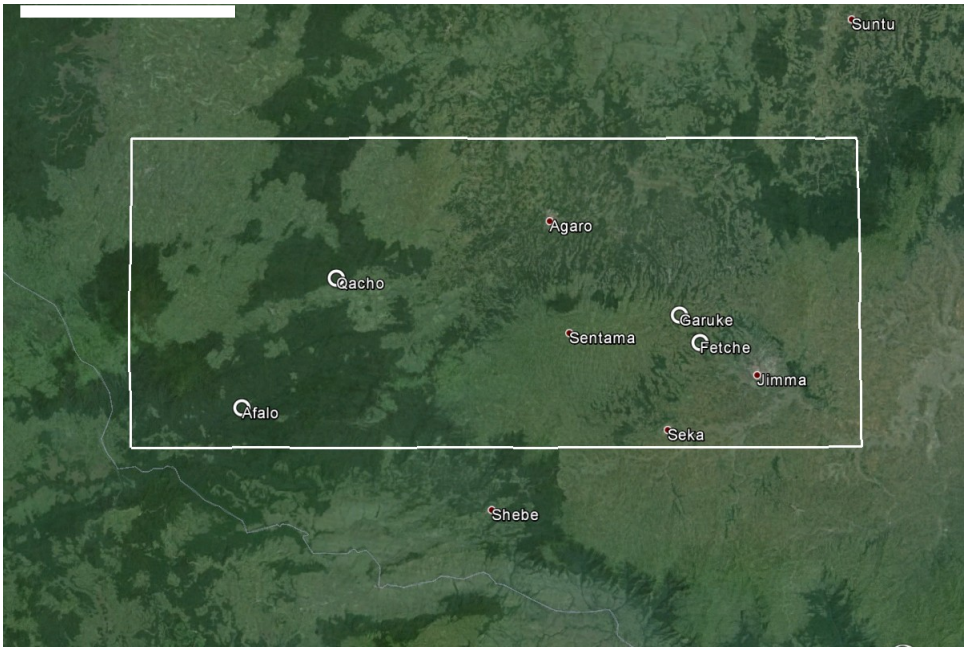


Figure 1.4 Location of the study area: **A**. in Ethiopia and **B**. in the Southwestern Highlands of Ethiopia. North up.

A

B

Figure 1.5 Semi-forest coffee study sites: **A**. Garuke and **B**. Fetche. North up.

A

B

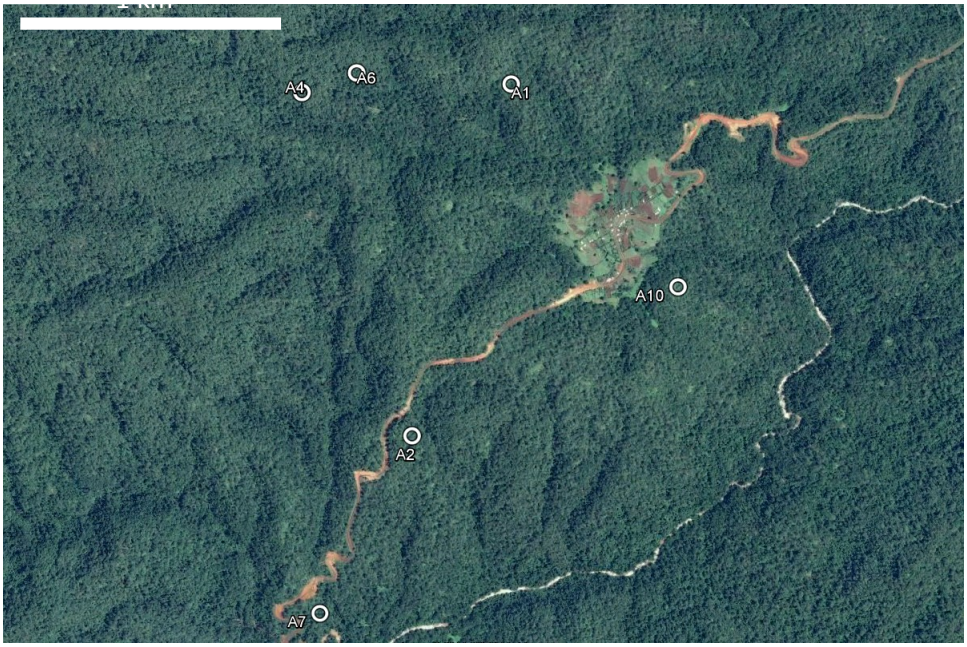


Figure 1.6 Forest coffee study sites: **A**. Qacho and **B**. Afalo. North up.

Outline of the thesis

This thesis consists of seven chapters.

CHAPTER 2: addresses the question: “Do Afromontane coffee forest fragmentation and coffee management intensity affect the level and structuring of the genetic diversity of wild Arabica coffee; and does the introduction of CBD-resistant cultivars pose a threat to the integrity of the wild Arabica gene pool?” Genetic diversity and degree of introgression is presented for 11 coffee forests stands.

CHAPTER 3: addresses the question whether forest management and fragmentation affect pollinator diversity. Has management intensity resulted in reduced pollinator abundance and diversity in SFC systems, compared to FC systems? It discusses the implication of pollinator abundance and diversity on Arabica coffee productivity.

CHAPTER 4: addresses the question whether management intensity affects matting patterns and pollen dispersal within fragmented coffee populations. Has management intensity resulted in reduced gene flow and outcrossing rates in highly managed forest fragments (SFC systems) compared to natural large forests (FC systems)? Is there a significant fine scale spatial genetic structure in wild coffee populations in FC systems, as compared to SFC systems? Is there higher transmission of genetic diversity from parent to offspring in wild coffee populations in FC systems, as compared to SFC systems?

CHAPTER 5: asks whether increasing forest fragmentation and forest management intensity cause pollen limitation in *C. arabica*. It shows the effect of flower manipulation on fruit and seed set; compares the degree of outcross and self pollen limitation, reproductive assurance, and autofertility in two contrasting coffee management systems (FC vs. SFC).

CHAPTER 6: asks how forest fragmentation and forest management intensity affect cup quality of *C. arabica*. It quantifies the effect of genotype, and growing conditions (soil physicochemical characteristics) on cup quality of *C. arabica* beans.

CHAPTER 7: gives an overview of the main results of the study followed by discussion and conclusions, guidelines for *in situ* conservation of wild Arabica coffee genepool and shortcomings of this study and research perspectives.

CHAPTER 2:

GENETIC VARIATION AND RISKS OF INTROGRESSION IN THE WILD *COFFEA ARABICA* GENE POOL IN SOUTHWESTERN ETHIOPIAN MONTANE RAINFORESTS

This chapter is adapted from:

Aerts R, **Berecha G**, Gijbels P, Vandepitte K, Van Glabeke S, Muys B, Roldan-Ruiz, I and Honnay O (2013) Genetic variation and risks of introgression in the wild Coffea arabica gene pool in southwestern Ethiopian montane rainforests. *Evolutionary Applications* **6**: 243-252.

2. 1 SUMMARY

The montane rainforests of SW Ethiopia are the primary centre of diversity of *Coffea arabica* and the origin of all Arabica coffee cultivated worldwide. This wild gene pool is potentially threatened by forest fragmentation and degradation, and by introgressive hybridisation with locally improved coffee varieties. We genotyped 703 coffee shrubs from unmanaged and managed coffee populations, using 24 microsatellite loci. Additionally, we genotyped 90 individuals representing 23 Ethiopian cultivars resistant to Coffee Berry Disease (CBD). We determined population genetic diversity, genetic structure, and admixture of cultivar alleles in the *in situ* gene pool. We found strong genetic differentiation between managed and unmanaged coffee populations, but without significant differences in within-population genetic diversity. The widespread planting of coffee seedlings including CBD-resistant cultivars most likely offsets losses of genetic variation attributable to genetic drift and inbreeding. Mixing cultivars with original coffee genotypes, however, leaves ample opportunity for hybridisation and encroachment of the original coffee gene pool, which already shows signs of admixture. *In situ* conservation of the wild gene pool of *C. arabica* must therefore focus on limiting coffee production in the remaining wild populations, as intensification threatens the genetic integrity of the gene pool by exposing wild genotypes to cultivars.

Keywords

Admixture, Afromontane rainforest, coffee, crop wild relative, ecosystem services, genetic erosion

2. 2 INTRODUCTION

To improve quality, achieve higher yields, or create pest and disease resistant or stress tolerant varieties of crops, plant breeders often utilize crop wild relatives (CWR's) (Hoisington *et al.* 1999; Heywood *et al.* 2007; Lashermes *et al.* 2011). CWR's are progenitors of crops and wild plant taxa that have relatively close genetic relationships to crops, but that are not domesticated themselves (Meilleur and Hodgkin 2004; Maxted *et al.* 2006). CWR's may possess desirable characteristics that can be used to improve existing crops (Gur and Zamir 2004; Fernie *et al.* 2006; Maxted *et al.* 2007; Takeda and Matsuoka 2008). In particular in light of global climatic change, sustained agricultural production may increasingly rely on the genetic enhancement of crops using the diverse germplasm of CWRs (Heywood *et al.* 2007; Tester and Langridge 2010; Ford-Lloyd *et al.* 2011; Foley *et al.* 2011), and for that reason, the *in situ* conservation of the genetic diversity of CWRs is an important but often undervalued challenge (Mercer and Perales 2010; Honnay *et al.* 2012).

Arabica coffee is one of the world's most valuable agricultural commodities, accounting for two-thirds of the global coffee market (Labouisse *et al.* 2008). Despite the currently wide geographic range of arabica coffee cultivation, the number of cultivars used is very small: mainly *Coffea arabica* var. *typica*, *C. arabica* var. *bourbon* and hybrids of the two (Labouisse *et al.* 2008). The narrow genetic base of those cultivars (Anthony *et al.* 2002) has resulted in a crop with homogenous agronomic behaviour (Lashermes *et al.* 2009), but also with a high susceptibility to biotic and climatic hazards (Labouisse *et al.* 2008; Jaramillo *et al.* 2011), and a low adaptability in response to environmental changes or changing market demands.

The closest wild relative of cultivated Arabica coffee is wild *Coffea arabica*, which has its origin and centre of diversity in southwestern Ethiopia (Anthony *et al.* 2001; 2002). Wild Arabica coffee is a unique potential source of genetic diversity for selection and breeding of enhanced arabica cultivars, including varieties with low caffeine content, increased yields, or increased resistance to pests and pathogens such as Coffee Berry Disease (CBD, caused by *Colletotrichum kahawae*), coffee rust (caused by *Hemileia vastatrix*), *Meloidogyne* root nematodes and the coffee berry borer

(*Hypothenemus hampei*) (Hein and Gatzweiler 2006; Silvestrini *et al.* 2007; Dessalegn *et al.* 2008; Boisseau *et al.* 2009). Despite its importance for the global coffee industry and for the livelihood of rural communities depending on coffee cultivation, the status of the wild gene pool of Arabica coffee is largely unknown and potentially threatened (Labouisse *et al.* 2008), a fate shared with many other CWRs in the world (Heywood *et al.* 2007).

Two major anthropogenic processes may potentially threaten the diversity and integrity of the gene pool of wild *Coffea arabica*: 1) the fragmentation and intensive management of the natural Afromontane rainforests, and 2) the large scale introduction of improved coffee varieties in natural coffee stands. First, as elsewhere in the tropics (Ahrends *et al.* 2010; De Fries *et al.* 2010), forest conversion to agriculture and other land uses related to urban population growth have resulted in the fragmentation of the Ethiopian montane forest (Gole *et al.* 2008). Furthermore, traditional forest coffee production practices in Ethiopia also alter forest structure and plant communities (Schmitt *et al.* 2009; Aerts *et al.* 2011). The intensity of management varies between so-called forest coffee (FC) systems, which undergo little or no intervention, and semi-forest coffee (SFC) systems, in which herbs, shrubs (other than coffee) and emerging tree seedlings in the understory are removed annually, the upper canopy is selectively thinned and coffee saplings are locally planted (Senbeta and Denich 2006; Schmitt *et al.* 2009; Aerts *et al.* 2011). Both forest fragmentation and forest degradation can have a negative impact on the genetic diversity of forest plant species through increased genetic drift, reduced gene flow, and alteration of mating patterns resulting in increased inbreeding (Young *et al.* 1996; Honnay *et al.* 2005; Eckert *et al.* 2010). Second, the widespread planting, since the 1970s, of a restricted set of locally improved coffee varieties, mainly genotypes resistant to Coffee Berry Disease (CBD), in the forest and its surroundings may result in the replacement of a part of the wild gene pool with a small number of domesticated alleles (Ellstrand *et al.* 1999; Becker *et al.* 2006; Hooftman *et al.* 2007). This can result in loss of genetic variation from the original gene pool and may even have negative fitness consequences for the original populations (Ellstrand 2003).

The general aim of this study was to provide the first thorough assessment of population genetic diversity within the wild gene pool of *Coffea arabica* in its centre of origin, the southwestern Ethiopian montane rainforests. We addressed the following specific questions: (i) Is there genetic erosion of the wild Arabica gene pool in fragmented forests managed for coffee production, compared to continuous non-managed forests? (ii) Is the introduction of CBD-resistant genotypes posing a threat to the integrity of the wild Arabica gene pool? The answers to these questions should help the *in situ* conservation of arabica coffee genetic resources.

2. 3 MATERIALS AND METHODS

Study species

Coffea arabica L. (family Rubiaceae) is the only *Coffea* species occurring in Ethiopia and is geographically isolated from all other species in the genus (Silvestrini *et al.* 2007). It is a naturally occurring understory shrub of the Afromontane rainforest, a type of moist evergreen montane rainforest found in the southwestern highlands between 1500 and 2600m, with an annual rainfall between 700 and 1500mm (Friis 1992). The canopy of the Afromontane rainforest typically consists of a mixture of broad-leaved species 10-30m tall with emergent trees that may reach a height of 30-40m (Demissew *et al.* 2004). Wild coffee generally occurs between 1500 and 1900m, but cultivated plants are found over a much wider range, between 1000 and 2800m (Hedberg *et al.* 2003; Gole *et al.* 2008). Flowering is induced by rains, with a short annual period of synchronous flowering usually in January. The species is self-compatible and mainly insect-pollinated, in Ethiopia typically by bees, which are attracted to the nectar (Fichtl and Admasu 1994). *Coffea arabica* fruits take about one year to reach maturity and are dispersed by birds, bats, monkeys, rodents and humans. Its population density varies by forest management intensity, with on average 3900 individuals (≥ 0.5m in height and dbh ≥ 2cm) ha^{-1} in the FC system compared to 18,500 individuals ha^{-1} in the SFC system (Schmitt *et al.* 2009). Within the genus, it is the only allotetraploid (2n = 4x = 44),

formed by relatively recent natural hybridization between *C. canophora* and *C. eugenioides* (Lashermes *et al.* 1999). The recent origin and self-fertilization of *C. arabica* probably contribute to its relatively low genetic diversity compared to diploid *Coffea* species (Lashermes *et al.* 2000).

Sample collection and DNA extraction

Coffee leaf samples were collected in eleven coffee stands in montane rainforest in the Jimma zone of Oromia region in SW Ethiopia (Table 2.1, Fig 2.1). Six stands were located in the remote Gera sector of the Belete-Gera National Forest Priority Area (NFPA) and were classified as forest coffee (FC). These stands showed no or only few signs of forest management. Five other stands were located in forest fragments (1-20ha in size) which are managed as SFC since the 1970s and which are located in the coffee-producing agricultural landscape east of the NFPA. All these forest fragments showed clear evidence of tree thinning, understory removal and locally, of coffee planting activities (including CBD-resistant cultivars), as revealed, for instance, by the high density and regular spacing of coffee plants (Aerts *et al.* 2011).

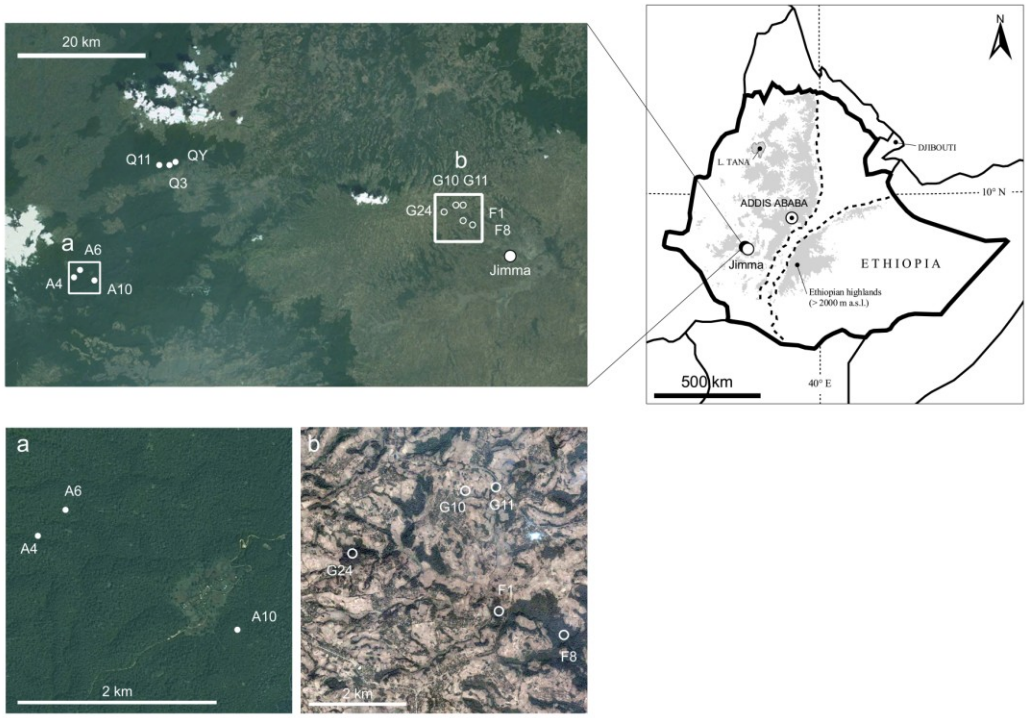


Figure 2.1 Afromontane rainforests in Southwest-Ethiopia and sampled *Coffea arabica* populations: forest coffee (closed circles) and semi-forest coffee (open circles). Insets show detail of the forest coffee (a) and of the semi-forest coffee landscape (b). Satellite imagery © 2012 DigitalGlobe, GeoEye and Cnes/Spot Image, via Google Earth.

In each stand, we established rectangular plots, containing approximately 65 coffee shrubs. Plots were at least 20m away from the edge in SFC forest fragments. Because of the difference in coffee density between forest stands, these plots varied in size between 12 and 225m^2. All shrubs within a plot were sampled for young leaf material, totalling 703 samples across 11 plots. In one forest stand (QY, Table 2.1), coffee density was very low and we sampled only 24 individuals. In two other stands (G11 and Q3), coffee density was higher than estimated when establishing the plots and we sampled 82 and 88 individuals, respectively.The coffee plants within a plot are further referred to as a population. This set was complemented with leaf samples of 90 individuals representing 23 different CBD-resistant varieties (741, 744, 7440, 7454, 7487, 74110, 74112, 74140, 74148, 74158, 74165, 754, 75227, Ababuna, Bunawashi,

Dessu, Gawe, Gesha, Melko-CH2, Me'oftu, Merdahereka, Wushwush and Yachi; hereafter called 'cultivars'), which were locally developed by the Ethiopian Institute of Agricultural Research from genotypes collected throughout the Ethiopian montane rainforests and which have been released between the late 1970s and 1990s. Leaf material was dried on silica gel. Before DNA-extraction, leaves were freeze-dried for 48 h and homogenized with a mill (Mixel Mill MM 200, Retsch®, Haan, Germany). Genomic DNA was extracted from 20mg homogenized leaf material using the NucleoSpin® Plant II kit (Machery-Nagel, Düren, Germany), with slight modifications of the standard CTAB protocol (we increased the incubation time during cell lysis to 60min at 65°C and used a two-step elution procedure incubated at 70°C for optimal recovery of bound nucleic acids).

Table 2.1. Location, sample size, molecular variance *MV*, expected heterzygosity corrected for sample size $H_{E,C}$ and population mean STRUCTURE cluster membership coefficients *Q* for eleven *Coffea arabica* stands and 23 cultivars in SW Ethiopia

							Q		
Stand	Lat (N)	Long (E)	Elev (m)	*N*	*MV*	$H_{E,C}$	I	II	SE
Forest coffee									
Afalo (A10)	7.6307	36.2241	1825	62	23.27	0.509	0.27	0.73	0.042
Afalo (A6)	7.6404	36.2092	1889	73	13.77	0.565	0.56	0.44	0.112
Afalo (A4)	7.6395	36.2067	1987	73	20.90	0.561	0.42	0.58	0.042
Qacha (Q11)	7.7868	36.3238	2108	63	15.82	0.600	0.67	0.33	0.086
Qacha (Q3)	7.7817	36.3313	1920	88	30.41	0.574	0.46	0.54	0.107
Qacha (QY)	7.7865	36.3432	1926	24	21.28	0.623	0.80	0.20	0.043
Semi-forest coffee									
Fetche (F1)	7.7144	36.7482	2085	60	25.06	0.552	0.78	0.22	0.003
Fetche (F8)	7.7106	36.7617	1908	61	14.80	0.554	0.81	0.19	0.031
Garuke (G10)	7.7368	36.7420	2025	57	17.63	0.560	0.69	0.31	0.069
Garuke (G11)	7.7373	36.7477	2040	82	23.99	0.547	0.57	0.43	0.138
Garuke (G24)	7.7256	36.7227	2062	60	20.01	0.500	0.62	0.38	0.144
Cultivars				90	23.25	0.621	0.58	0.42	0.053

SSR genotyping

Twenty-four microsatellites (SSRs) were amplified in six multiplex PCRs (Appendix Table 2.1) using a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems®,

CA, USA) and a total sample volume of 10µL containing 5µL Qiagen® Multiplex PCR Master Mix (Qiagen, Valencia, CA), 2µL sample/template DNA and 0.2µL of each primer (reverse and forward, 10µM) in the multiplex combination complemented with RNase-free Milli-Q water. The multiplexes had equal thermocycling profiles with an initial *Taq* DNA polymerase heat-activation step at 95°C for 15min; 25 cycles of 30s at 94°C (denaturation step), 90s at 57°C (annealing step) and 60s at 72°C (extension step); and a final extension step of 30min at 60°C. Then, 1µL of the PCR reaction was added to a solution of 8.8µL formamide and 0.2µL of the Applied Biosystems GeneScan^{TM} 500 LIZ® size standard. Sized fragments were scored using GeneMapper® v4.0 (Applied Biosystems).

Genetic data analysis

The allotetraploid nature of the *Coffea arabica* genome limits the flexibility of the data analysis. We adopted two parallel approaches, one based on the co-dominantly scored data and allowing allele copy number ambiguity, and a second based on the scoring of each individual allele as present or absent, resulting in a dominantly scored dataset comparable to the output of an Amplified Fragment Length Polymorphism marker approach. We used the R package POLYSAT (Clark and Jasieniuk 2011) as a central data handling facility, i.e. for importing the SSR data from the GeneMapper® software and for converting the data. To assess the resolution of the microsatellite marker set we discriminated distinct multilocus genotypes (MLGs).

Genetic diversity and population differentiation

Population genetic diversity was quantified using the expected heterozygosity corrected for sample size ($H_{E,C}$), and the molecular variance (*MV*) based on the within population sum of squares (*SSWP*) and calculated as $SSWP \times (n\text{-}1)^{-1}$. Among population genetic differentiation (Φ_{PT}) was calculated based on Euclidian genetic distances (Huff *et al.* 1993). $H_{E,C}$ is based on the tetraploid data set and was calculated in ATETRA 1.2.a (Van Puyvelde *et al.* 2010) whereas *MV* and Φ_{PT} resulted from a hierarchical analysis of molecular variance (AMOVA) approach on the dominantly scored data set as performed in GENALEX 6.41 (Peakall and Smouse 2006). For the AMOVA, we used the coffee

production system (FC vs. SFC) as the regional grouping variable. Genetic differentiation was further assessed using principal coordinates analyses (PCoA) calculated in GENALEX on the pairwise Φ_{PT} matrix. Effects of coffee forest management intensity (FC vs. SFC) on population genetic diversity were analysed using Wilcoxon-Mann-Whitney *U* tests. To test whether the mean genetic diversities recorded in FC and SFC populations differed from the diversity in the cultivar population, we used one-sample t-tests. Finally, also the average pairwise genetic differentiation (Φ_{PT}) between populations in FC stands was compared with the differentiation between populations in SFC stands, using a Wilcoxon-Mann-Whitney U test. Statistical analyses were performed in SPSS 15.0 (SPSS Inc., Chicago, IL).

Bayesian analysis of population structure

To investigate the presence of alleles from the cultivar gene pool across the coffee populations, genetic structure was assessed using Bayesian clustering analysis implemented in STRUCTURE 2.3.3 (Pritchard *et al.* 2000; Falush *et al.* 2007; Pritchard *et al.* 2010). STRUCTURE was run five times at K = 1–9 applying 10 000 burn-in cycles and 50 000 Markov Chain Monte Carlo (MCMC) iterations. Correlated allele frequencies and admixture were assumed. The degree of admixture was inferred from the data using an initial value of α = 1.0 and a maximum of 10. The value of K that best fitted our data was selected using the estimated log probability of data Pr(X|K) and the derived ΔK statistic (Evanno *et al.* 2005). We used CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) to match the five STRUCTURE solutions and calculate average ancestry estimates, given as estimated membership coefficients Q for each individual and each population, in each of K clusters.

Analysis of genome-wide admixture

We assessed genome-wide admixture of alleles from the cultivar gene pool into the FC and SFC populations using a hybrid index or admixture coefficient (Gompert and Buerkle 2009), as calculated in the R package INTROGRESS (Gompert and Buerkle 2010). The SSR data of the populations A10 and Q3 and of the cultivar population were used as wild and cultivar parental data, respectively, and the SSR data of the remaining

FC and all SFC populations were entered as potentially admixed individuals. For this analysis, A10 and Q3 were selected as pure wild populations because these were the only populations where the owners guaranteed they had not planted any cultivars, and thus, where the introduction of coffee plants had not taken place. The est.h function of INTROGRESS was applied to calculate a maximum likelihood hybrid index estimate *h* for each potentially admixed individual (Gompert and Buerkle 2010). We compared hybrid index means of FC and SFC using the independent-samples t-test.

2. 4 RESULTS

Genetic diversity and differentiation

The 24 SSRs in the six multiplex combinations yielded a total of 159 alleles. The number of alleles ranged from 2 to 19 per locus. Only eleven individuals were assigned to four non-unique MLGs across populations. The expected heterozygosity corrected for sample size ($H_{E,C}$) and the molecular variance (*MV*) did not differ significantly between FC ($H_{E,C}$ = 0.571, SE 0.016; *MV* = 20.91, SE 2.40) and SFC populations ($H_{E,C}$ = 0.552, SE 0.014; *MV* = 20.30, SE 1.92) ($H_{E,C}$: *U* =10, *P* = 0.429; *MV*: *U* =15, *P* = 1.000). The sample of CBD-resistant varieties was genetically more diverse ($H_{E,C}$ = 0.621; *MV* = 23.25) than both the FC and SFC populations but this difference was only significant for $H_{E,C}$ (t_{10} = -5.90, *P* < 0.001). Overall among-population genetic differentiation was high (Φ_{PT} = 0.186, *P* < 0.001), with a genetic differentiation of 0.033 (Φ_{RT}) between production systems (Table 2.3). Genetic differentiation among populations was significantly higher for the SFC (Φ_{PT} = 0.176, SE 0.018) than for the FC (Φ_{PT} = 0.131, SE 0.014) populations (*U* = 38, *P* = 0.040).

Table 2.3. Hierarchical analysis of molecular variance of 159 alleles at 24 microsatellite loci for 703 *Coffea arabica* individuals distributed in 11 stands and two coffee production systems, forest coffee (FC) and semi-forest coffee (SFC), in SW Ethiopia

Source	*df*	*SS*	*MS*	*EV*	*%MV*	Φ-statistic		*P*
among systems (FC-SFC)	1	586.0	586.0	0.85	3.27	Φ_{RT}	0.033	0.001
among populations (stands)	9	2452.7	272.5	3.97	15.36	Φ_{PR}	0.159	0.001
within populations (stands)	696	14623.5	21.0	21.01	81.37	Φ_{PT}	0.186	0.001
Total	706	17662.2		25.82				

For each source of variation the following is given: the number of degrees of freedom (*df*), the sum of squared difference to the mean (*SS*) and the mean sum of squares (*MS*), the estimated variance (*EV*), the percentage of total molecular variance (*%MV*), the Φ-statistic and the associated probability.

Populations from FC and SFC clustered at opposite ends in the PCoA with some overlap among clusters (e.g. F1, Q3) (Fig. 2.2).

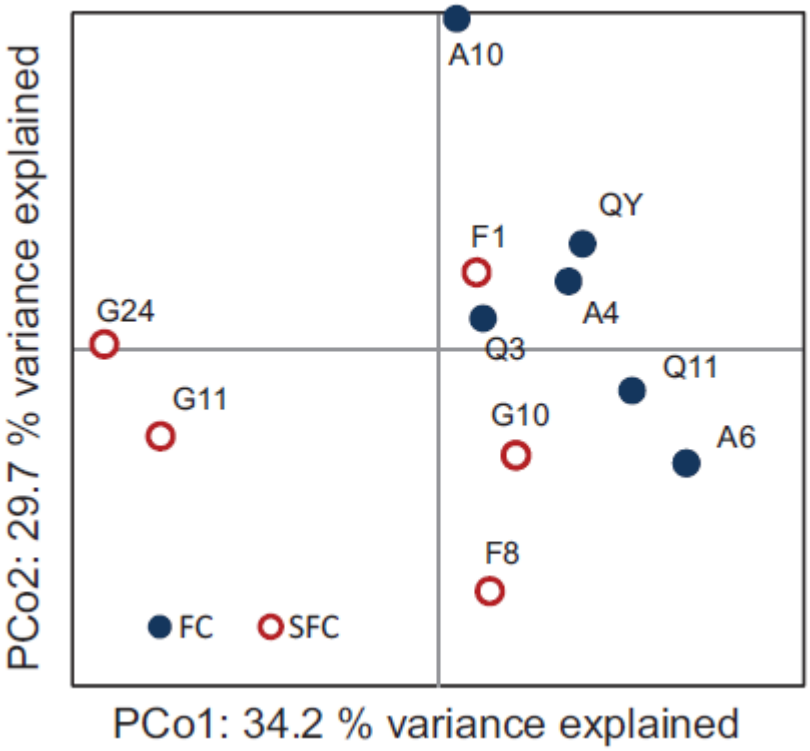


Figure 2.2 Principal coordinates (PCo) plot based on Φ_{PT} calculated with 24 SSR markers for *Coffea arabica*, demonstrating population genetic differentiation between forest coffee (closed circles) and semi-forest coffee systems (open circles).

Genetic structure and admixture

The log probability of data increased with increasing *K* but was less pronounced when *K* > 2. This, together with the fact that the ΔK statistic reached its maximum at *K* = 2 (Fig. 2.3), suggested the existence of two clusters.

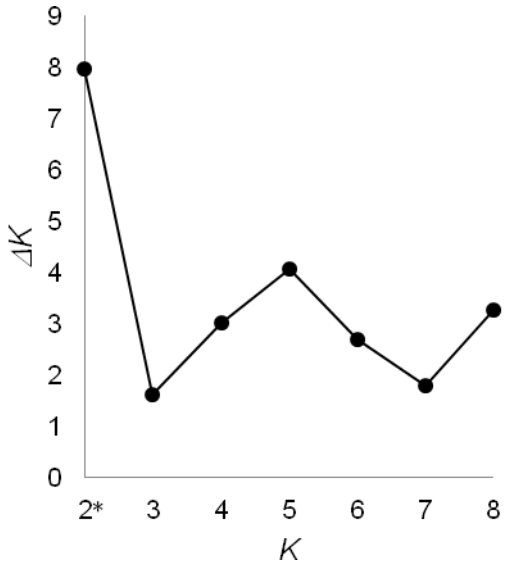


Figure 2. 3 *ΔK* statistic for detection of the true number of groups *K** or the value of *K* that best fit the data

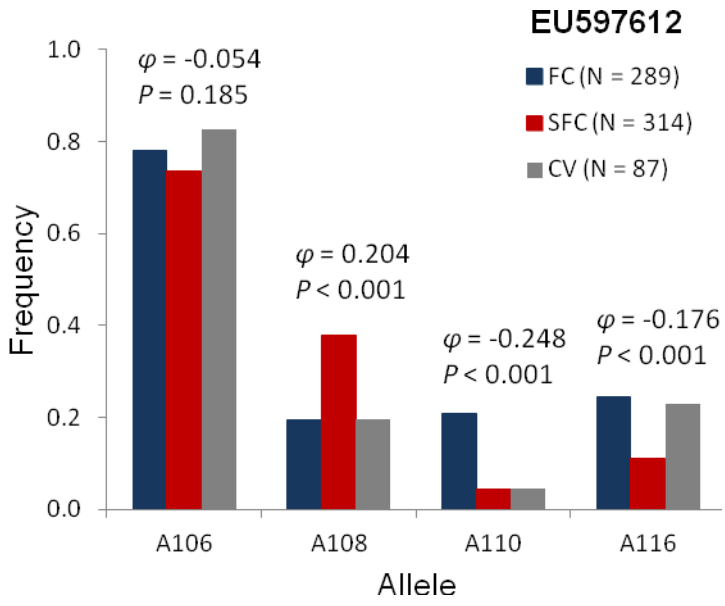


Figure 2.4 Relative frequency distribution of alleles for *Coffea arabica* microsattelite EU597612 (samples with missing values for this SSR were omitted), illustrating cryptic genetic erosion in the *in situ* arabica gene pool. The same four alleles are present in FC and SFC, but within the SFC two of these four alleles are rare. The *φ*-statistic and probability *P* show a significant association of two alleles to FC and one to SFC.

The genotypes assigned to cluster I were by far the most widespread and dominated five SFC populations and two FC populations. Genotypes assigned to cluster II were dominant in the remote FC population A10. One SFC population (G11), three FC populations (A6, A4, Q3) and the cultivars had comparable proportions of individuals assigned to either cluster I or II, as well as individuals with an admixed genotype, i.e. assigned to both genetic clusters with comparable probabilities (Table 2.1; Fig. 2.5).

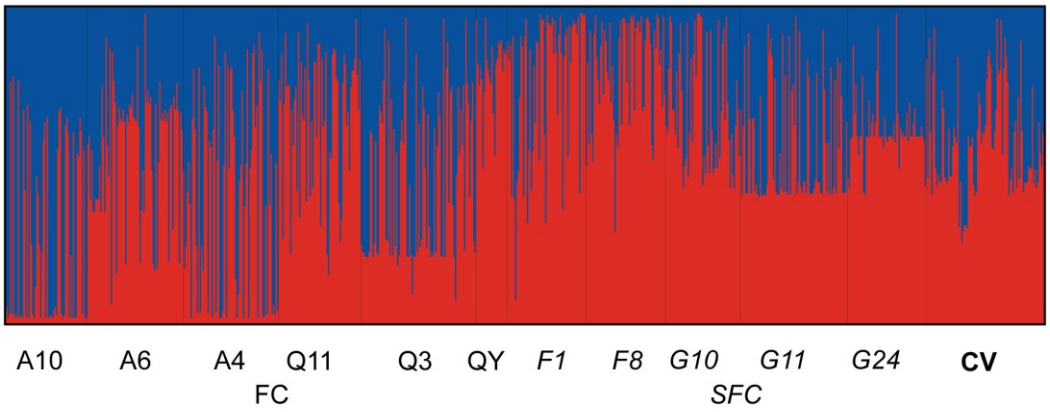


Figure 2.5 Population structure of *Coffea arabica* based on STRUCTURE analysis of 24 SSR markers for forest coffee (*N* = 383, 6 populations), semi-forest coffee (*N* =320, 5 populations) and cultivar (*N* = 90) samples for *K* = 2 clusters (Fig. 2.3). Individuals are represented by columns, with colours showing the average proportion (*R* = 5 runs) of their genome assigned to the different clusters, demonstrating the prevalence of a genotype associated to the CBD-resistant gene pool in the SFC populations.

The admixture coefficients or hybrid indices varied between 0 (pure 'wild') and 1 (pure 'cultivar'). In the SFC populations, hybrid indices were predominantly high, with $h > 0.50$ for 86.3% of the samples. In the FC populations, hybrid indices were low, with $h < 0.50$ for 82.4% of the samples (Fig. 2.6). The mean hybrid index was significantly higher in the SFC (mean h_{SFC} = 0.74, SE 0.012; h_{FC} = 0.30; SE 0.015) (t_{551} = 22.4, $P < 0.001$). These results indicate that alleles from the cultivar gene pool are more prevalent in the SFC than in the FC populations, and that most individuals in the SFC populations have

more alleles from the cultivar gene pool than alleles from the wild gene pool in their genome.

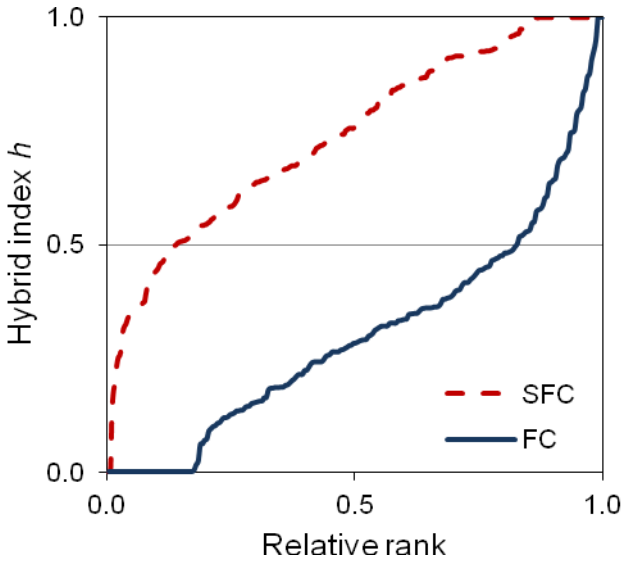


Figure 2.6 Maximum likelihood hybrid index estimates *h* for 320 *Coffea arabica* individuals from 5 semi-forest coffee populations and for 233 *Coffea arabica* individuals from 4 forest coffee populations in SW Ethiopia. The *h* index or admixture coefficient, based on frequencies for 159 alleles, a parental population of *N* = 150 wild individuals (from populations A10 and Q3) and a parental population of *N* = 90 specimens from 23 CBD-resistant varieties, gives the fraction of the genome shared with the cultivated varieties for each individual.

2. 5 DISCUSSION

Genetic diversity and cryptic genetic erosion

Although SFC-management is associated with major changes in forest structure and shrub and canopy species composition (Schmitt *et al.* 2009; Aerts *et al.* 2011), it had no negative impact on coffee genetic diversity within populations ($H_{E,C}$, *MV*). This was surprising because negative effects on effective population size and genetic diversity of

C. arabica populations could have been expected. Changes in forest microclimate and in availability of nesting sites for insect pollinators, for instance, may have negatively affected pollinator diversity (Klein *et al.* 2008), reducing cross pollination (Klein *et al.* 2003), increasing selfing and inbreeding (Eckert *et al.* 2009), and reducing progeny vigour and effective population size (Honnay and Jacquemyn 2007). As SFC systems are already managed as such for some generations (~40 years), it is unlikely that expected genetic changes still have to become apparent. More plausible, and in agreement with our other results, is that farmers have been introducing new genotypes from elsewhere, both CBD-resistant genotypes provided by local authorities and wild genotypes from neighbouring sites, compensating for the eventual loss of genetic variation through genetic drift and inbreeding.

The introduction of genetically diverse coffee genotypes from elsewhere into SFC populations by coffee farmers most likely represents a farmer-mediated evolutionary force. This is supported by the higher genetic differentiation (Φ_{PT}) among SFC populations than among FC populations. The conspicuous presence of alleles from the pool of introduced CBD-resistant genotypes in almost all SFC populations (high *h*, Fig. 2.6) also suggests that genetic diversity in the original coffee populations is being displaced, which is a form of cryptic genetic erosion (see Fig. 2.4). The local introduction of CBD-resistant genotypes in the SFC area since the 1970s probably facilitated later hybridization through gene flow by pollen (Papa and Gepts 2003). We can, however, not exclude that regional differences contributed to the higher genetic differentiation within the SFC region but we expect that their effect is limited. First, given that FC populations have been sampled over a much larger area (25×25km², Fig. 2.1) than the SFC populations (5×5km²), this higher genetic differentiation is unlikely to be caused through isolation-by-distance or by greater environmental variation among SFC populations. Second, gene flow through pollen or seed between SFC populations may have been impeded due to the more fragmented nature of the agricultural landscape near Jimma town, increasing the among population genetic differentiation. If the latter was the case, however, we expect genetic differentiation to be paralleled by loss of genetic diversity through genetic drift, which was not observed in the SFC populations.

Introduction of alleles from CBD-resistant varieties

Gene flow from domesticated crops to wild relatives poses an important potential threat to CWRs, as repeated occurrences of hybridisation may lead to the loss of the genetic integrity of the wild species (introgression), which becomes assimilated into the cultivar. This process has already increased the risk of extinction of the wild relatives of two of the world's 13 most important crops, rice (*Ozyra sativa*) and cotton (*Gossypium hirsutum*) (Ellstrand *et al.* 1999). Evidence of introgression from modern hybrid crop varieties into wild populations and populations of locally domesticated landraces is rapidly emerging (e.g. Sørensen *et al.* 2007; Bitocchi *et al.* 2009; Arnaud *et al.* 2010; Arrigo *et al.* 2011; Kwit *et al.* 2011), warning ecologists and plant breeders about the latent extinction of wild relative populations. The high genetic variation within the group of introduced CBD-resistant genotypes in this study (Table 2.1) strongly suggests that the improved varieties that have been released in Ethiopia did not yet undergo an extreme process of breeding and selection, and that the process of domestication so far was focussed on the capture and multiplication of genotypes with desirable traits. So far, coffee breeding in Ethiopia has been less intensive than for other crops such as maize, which underwent a rigorous process of breeding and selection, yielding elite breeding pools with very little of the genetic diversity found in the maize wild relatives (Ortiz *et al.* 2010). In general, genetic domestication bottlenecks seem to be more limited in perennial fruit crops than in annual crops (Miller and Gross 2011), although commercial coffee cultivars grown in central America or Asia show important losses of genetic diversity (Lashermes *et al.* 1996; López-Gartner *et al.* 2009).

Our results show the striking presence of alleles from the CBD-resistant gene pool in all SFC populations, and to a much lesser extent in some FC populations. This suggests the possibility that introgression from recently introduced cultivars may be common in wild populations. Although the process of introgression may be slower in long-lived or clonal plants, such as coffee, post-zygotic barriers to hybridization may be weaker than in domesticated annuals such cereals, where hybrid seedlings have been shown to be maladapted to wild environments (McKey *et al.* 2010). The CBD-resistant cultivars were released starting in the 1970s, providing ample opportunities for

hybridization, given that the generation time for coffee is ca. three years. Hybridization between cultivars and wild individuals has been reported in a number of long-lived perennials, such as grapevine (*Vitis vinifera*, De Andrés *et al.* 2012), wild almond (*Prunus orientalis*, Delplancke *et al.* 2012) and wild apple (*Malus sylvestris*, Coart *et al.* 2003). However, we cannot rule out that the presence of these alleles in wild populations may simply reflect the shared ancestry between introduced and wild genotypes. CBD-resistant genotypes are derived from forest coffee trees from different parts of Ethiopia, and at least some of the alleles shared between the cultivar gene pool and the SFC are expected to be identical by descent. Further research should focus in depth on gene flow between wild coffee and introduced cultivars, for example through parentage analysis (De Andrés *et al.* 2012).

2. 6 CONCLUSIONS

Our results clearly show that SFC populations are genetically more similar to the pool of introduced CBD-resistant genotypes than FC populations, but that SFC populations are not less diverse than FC populations. These patterns can be explained by the anthropogenic introduction of genotypes (both wild genotypes and CBD-resistant cultivars) in SFC populations. Although we cannot provide direct evidence for hybridisation and introgression, the practice of large scale planting of CBD-resistant genotypes by local farmers in SFC systems, intimately mixed with original coffee genotypes, offers ample opportunity for the exchange of alleles and the encroachment of the original coffee gene pool. Only the few remaining large forests with a FC cultivation system seem to be safe from the introduction of CBD-resistant cultivars so far. To ensure the *in situ* conservation of Arabica coffee genetic resources in Ethiopia, we recommend to: (i) avoid establishing plantations with foreign coffee cultivars in the centre of origin of *C. arabica*; (ii) allow only the use of Ethiopian cultivars that did not undergo a very strict process of selection in the SFC systems; and (iii) protect a sufficiently large area of low-intensity FC systems. The latter can probably only be realized by establishing buffer zones of SFC surrounding more strict reserves of FC in the last remaining large forests blocks in the region.

CHAPTER 3:

INCREASING FRAGMENTATION AND MANAGEMENT OF ETHIOPIAN MOIST EVERGREEN COFFEE FORESTS RESULTS IN COMPOSITIONAL SHIFTS OF INSECT COMMUNITIES VISITING WILD ARABICA COFFEE FLOWERS

This chapter is under review:

Berecha G, Aerts R, Muys B and Honnay O (2014) Increasing fragmentation and management of Ethiopian moist evergreen coffee forests results in compositional shifts of insect communities visiting wild Arabica coffee flowers. *Submitted to Environmental Management Journal.*

3.1 SUMMARY

Coffea arabica is an indigenous understory shrub of the moist evergreen Afromontane forest of SW-Ethiopia. Coffee cultivation here occurs under different forest management intensities, ranging from almost no intervention in the 'forest coffee' system to far-reaching interventions that include the removal of competing shrubs and selective thinning of the upper canopy in the 'semi-forest coffee' system. We investigated whether increasing forest management and fragmentation result in potential impacts upon coffee pollination services through examining shifts in insect communities that visit coffee flowers. Overall, we netted 2976 insect individuals on *C. arabica* flowers, belonging to sixteen taxonomic groups, comprising 10 insect orders. Taxonomic richness of the flower visiting insects significantly decreased and pollinator community changed with increasing forest management and fragmentation. The relative abundance of honey bees significantly increased with increasing forest management and fragmentation, likely resulting from the introduction of bee hives in the most intensively managed forests. The impoverishment of the insect communities through increased forest management and fragmentation potentially decreases the resilience of the coffee production system as pollination increasingly relies on honey bees alone. This may negatively affect coffee productivity in the long term as pollination services by managed honey bees are expected to decline under current climate change scenarios.

Keywords: Pollinating insects, pollinator community, *Coffea arabica*, forest management, honeybee, forest fragmentation

3. 2 INTRODUCTION

Human-mediated pollinator losses may have considerable impact on the productivity of cultivated crops (Garibaldi *et al.* 2011). Almost 70% of the global crops depend to some extent on animal pollination (Klein *et al.* 2007), representing a total economic value of ca. €153 billion (Gallai *et al.* 2009). Decreases in yield due to disturbed pollination processes may cause the need to intensify the cultivated land area, increasing rates of habitat loss and, in turn, causing further pollinator losses (Steffan-Dewenter *et al.* 2005; Tscharntke *et al.* 2005; Klein *et al.* 2007; Aizen *et al.* 2009). To maintain productive cropping systems and to achieve environmental sustainability, an accurate understanding of how human disturbance affects pollinator diversity and crop visitation rates is required. Growing empirical evidence shows considerable losses of pollinators in many regions of the world, with the strongest evidence coming from Europe and North America (Potts *et al.* 2010). The diversity and abundance of pollinators may decline due to simultaneous and synergistic effects of several drivers, including agricultural intensification (Briggs *et al.* 2013), habitat loss and fragmentation (Hendrickx *et al.* 2007; Winfree *et al.* 2009), environmental pollution resulting from increased pesticide application (Vidau *et al.* 2011; Gill *et al.* 2012), invasion by non-indigenous competitors, pollinators and herbivores (Traveset and Richardson 2006; Badano and Vergara 2011), and decreased resource availability and diversity (Biesmeijer *et al.* 2006).

Pollinator declines may have a particularly strong impact in tropical regions where most plant species are pollinator dependent (Bawa *et al.* 1990; Ollerton *et al.* 2011). Tropical regions encompass much of the world's biodiversity hot spots (Myers *et al.* 2000) and contribute substantially to world agriculture by producing economically important crops such as coffee and cocoa (O'Brien and Kinnaird 2003; Donald 2004). These crops are traditionally grown in agroforestry systems that resemble natural forest (Perfecto and Vandermeer 2008) but intensification of these systems is expected to have negative effects on the abundance and diversity of pollinators, reducing pollination

services and, hence, crop productivity (Boreux *et al.* 2013b; De Beenhouwer *et al.* 2013).

Arabica coffee (*Coffea arabica*) plays a central role in the Ethiopian economy, contributing over 35 % to the total export (Labouisse *et al.* 2008). In Ethiopia, over 15 million people are directly or indirectly dependent on the coffee sector for their livelihood (Labouisse *et al.* 2008). Arabica coffee or highland coffee is a native species of the understory of moist evergreen Afromontane forests, and is endemic to southwestern Ethiopia (Anthony *et al.* 2001; 2002). Although *C. arabica* is self-compatible, many studies conducted in plantations or rustic coffee production systems in South America and Asia have demonstrated a significant increase (ranging from ca. 50 to 80%) in fruit yield in pollinator-rich environments (e.g., Roubik 2002; Klein *et al.* 2003a, b; Veddeler *et al.* 2008). In recent years, pollinator communities associated with *C. arabica* pollination have been well documented in the coffee growing regions of Asia and Latin America (e.g. Klein *et al.* 2003b; Veddeler *et al.* 2006; Klein *et al.* 2009; Jha and Vandermeer 2010; Badano and Vergara 2011; Peters and Carroll 2012). Veddeler *et al.* (2006) reported that 95% of flower visitors to coffee in Ecuador were social bees and less than 5% solitary bees. In Indonesian agroforestry coffee plantations70% of flower visitors were social bees and 30% solitary bees (Klein *et al.* 2003a; 2003b). So far, information regarding the diversity and community composition of pollinators of Arabica coffee in its Ethiopian center of origin and diversity is largely missing. Exceptions are rather anecdotal reports of highland honey bees (*Apis mellifera monticola*, (Smith 1965)) and solitary bee species (Martins 2007) pollinating Arabica coffee.

Like many other tropical forests, most of the moist evergreen Afromontane forests of Ethiopia have become extremely fragmented (Getahun *et al.* 2013), with deforestation rates up to 1.1% per year (FAO 2011). Furthermore, many of the remaining forests that harbor *C. arabica* are intensively managed for coffee cultivation (Aerts *et al.* 2011; Hundera *et al.* 2013a). The traditional coffee agroforestry production systems in this part of Ethiopia include the so-called “Forest coffee (FC)” and “Semi-forest coffee (SFC)” systems (Senbeta and Denich 2006). These traditional production systems differ in terms of management intensity (Aerts *et al.* 2011). Unlike FC, where

human intervention to increase coffee productivity is little or absent, SFC systems experience rigorous interventions which include the removal of competing understorey shrubs and selective thinning of the upper canopy to reduce crown closure while maintaining high crown cover (Schmitt *et al.* 2009; Aerts *et al.* 2011). It has already been shown that this intensive forest management has detrimental effects on tree species diversity of the Afromontane forest (Hundera *et al.* 2013a; Tadesse *et al.* 2014), on the diversity of associated taxa such as epiphytic orchids (Hundera *et al.* 2013b), and even on the genetic integrity of wild Arabica coffee populations (Aerts *et al.* 2013). Because coffee management practices have important consequences on forest structure and plant species composition, it can be expected that they also have a strong impact on pollinator abundance and their foraging behavior (Brosi *et al.* 2008; González-Varo *et al.* 2009; Brosi 2009; Jha and Vandermeer 2010; Boreux *et al.* 2013b).

The general aim of this study was to quantify the effects of forest management intensity and forest fragmentation on the coffee pollinator community in SW Ethiopian moist evergreen Afromontane forests. Therefore, we surveyed the taxonomic diversity of insects visiting coffee flowers of sites in both large and small intensively managed forest fragments, and compared these to the pollinator community of sites in a very large natural forest. Our specific objectives were:

1) To provide an account of the diversity and composition of the insect communities visiting coffee in the moist evergreen Afromontane forests of southwest Ethiopia; and

2) To quantify the effects of both forest management intensity and forest fragmentation on the taxonomic group diversity and community composition of coffee flower visiting insects.

3. 3 MATERIALS AND METHODS

Description of the study area

The study was conducted in 40 sampling sites in the moist evergreen Afromontane forest of southwest Ethiopia. Twenty sites were located in 20 isolated, small and intensively managed (SFC system) forest fragments (ca. 0.5-9ha in size) in the Manna district near the village of Garuke. We further refer to these sampling sites as small managed forest (SMF). Next, ten forest blocks, each with a size of c. 4ha, were randomly selected in one large (≥ 100ha), highly managed forest (SFC system) near the village of Fetche, also in the Manna district. These sampling sites are further referred to as large managed forest (LMF). Finally, we randomly selected 10 forest blocks of c. 4ha, in the large natural Gera forest (over 100,000ha) (FC system), in the Gera district. Despite the ongoing internal degradation and fragmentation, Gera forest is one of the last remaining, least disturbed moist evergreen Afromontane forests in the area (Hundera *et al.* 2013b). These sampling sites are further referred to as large natural forest (LNF). To minimize edge effects, forest blocks were established at least 200m from the edge in LNF. The minimal distance between forest blocks within LNF and LMF was 500m, but most were separated by more than 1000m. Characteristics of the managed (LMF, SMF) and unmanaged forest (LNF) in terms of the intensity of forest management is summarized in the Table 3.1.

Table 3.1 The type and intensity of coffee management practices in the two traditional coffee production systems in the southwest Ethiopia Afromontane forest (Senbeta and Denich 2006; Schmitt *et al.* 2009; Aerts *et al.* 2011; Hundera *et al.* 2013b).

Coffee management practices	SFC	FC
Rigorous slashing of undergrowth	High to very high	No
Planting of coffee seedlings	High	No
Tree cutting	High to very high	Little to No
Tree species richness	32 (ranging from 26-38 species per fragment)	44
Seedling density per ha	3,000	10,000
Stem density per ha	655	952

SFC= include large managed forest (LMF) amd small managed forest (SMF); FC= include large natural forest (LNF).

Pollinator sampling

Surveys of coffee flower visitors were conducted from the first week of January to the last week of March 2013, during the main coffee flowering period in the study area. Coffee flowering is ephemeral, triggered by the first rain showers. Flowers open just before dawn, and last for only 2-3 days. We surveyed all fully blooming coffee shrubs along a 20m x 50m transect that was established centrally in each forest fragment (for SMF) or forest block (for LMF and LNF). Because it was unfeasible to identify the species *in situ*, we followed a netting approach. Visiting insects were netted during sunny days between 9:00AM and 3:00PM. In each transect, all selected blooming coffee shrubs were netted for a duration of 25 minutes, using white sweep nets. Only species sitting on the coffee flowers/inflorescence were collected. The netted individuals were assigned to 16 major taxonomic groups (see Appendix Table 3.1).

Statistical analysis

We calculated true diversity N1 (the exponent of Shannon's diversity index H' or Hill's index N1 = $e^{H'}$) (Hill 1973) at each sampling site because N1 is relatively unaffected by species richness and tends to be independent of sample size. We also calculated beta diversity for each management type as a measure of the gradient of species turnover. To account for bias due to differences in sampling effort among forest management types (LNF, SMF, LMF), we performed sample–based rarefaction (Mao Tau species accumulation curves) and calculated incidence–based estimations of the number of taxonomic groups in each forest type (Chao2), using EstimateS v.9 (Colwell *et al.* 2012). To quantify the pollinator community composition at each sample site, non-metric multi-dimensional scaling (NMS) was performed on the pollinator abundance data. Multivariate differences in taxonomic composition between the three management types were tested with a multi-response permutation procedure (MRPP) in PCORD V. 5.31 (McCune and Mefford 2006). For statistical comparisons among forest management types, in terms of N1, NMS1 and NMS2, and abundance of taxonomic groups, we first verified the absence of spatial autocorrelation among the selected forest blocks in the LMF and LNF sampling sites, using a Mantel test. We used a geographic distance matrix, calculated from the coordinates of the center of each forest block, and a Sørensen distance matrix, calculated from abundance data of the identified taxonomic groups. None of the Mantel tests showed a significant isolation by distance relation ($P > 0.1$), and all samples from the forest blocks were therefore considered to be independent observations. The variables N1, NMS1 and NMS2 fulfilled normality assumptions and we consequently used one way ANOVA to compare forest management types with respect to different measures of pollinator diversity, followed by pairwise comparisons among types, using a Tukey test. These analyses were performed in SPSS v19 (SPSS Inc. 2011).

3. 4 RESULTS

We collected a total of 2976 individuals on *C. arabica* flowers, all of them insects (Appendix Table 3.1; Appendix Fig.3.1). The five most abundant taxonomic groups were honey bees (78% of all sampled individuals), butterflies (4.8%), hoverflies (4.2%), other bees (2.8%) and beetles (1.4%). We collected 1162 individuals belonging to all sixteen taxonomic groups in LNF; 970 individuals belonging to fourteen taxonomic groups in LMF; and 844 individuals belonging to thirteen taxonomic groups in SMF. In all the three forest management types, honey bees (*Apis mellifera* L) were the dominant flower visitors, and their relative abundance increased significantly with increasing fragmentation and forest management intensity (Fig. 3.1A; $F_{2,37}$ = 114.07, P<0.001; all pairwise comparison P<0.05). Honey bees accounted for 70, 80 and 92% of the total number of individuals caught in LNF, LMF and SMF, respectively. Butterfly abundance significantly decreased with increasing fragmentation and forest management intensity (Fig. 3.1B; $F_{2, 37}$ = 16.60, P<0.001; all pairwise comparison P < 0.05). The abundance of hoverflies followed the same trend ($F_{2, 37}$ = 50.54, P <0.0001). Other bee species were more abundant in LNF compared to LMF and SMF ($F_{2, 37}$ = 37.76, P <0.001), but there was no significant difference between LMF and SMF. We also detected higher abundance of beetles in LNF compared to SMF but there was no significant difference between LNF vs. LMF and LMF vs. SMF ($F_{2, 37}$ = 6.41, P = 0.004).

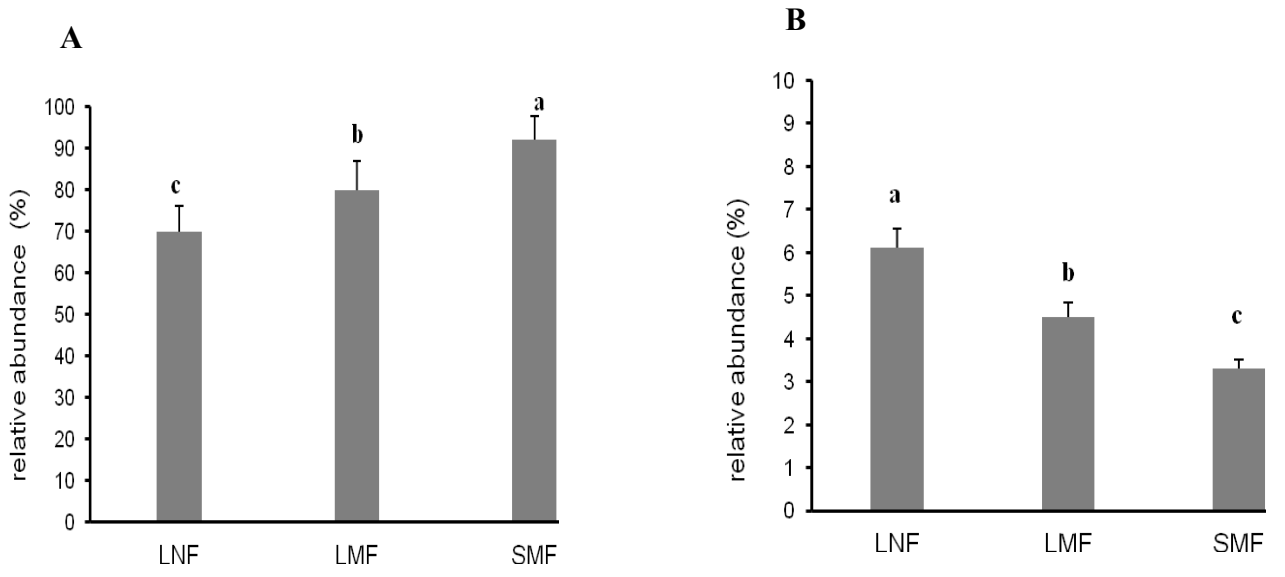


Figure 3.1 Effect of forest type on the relative abundance of *Apis mellifera* L. (A) and butterflies species (B) on flowering coffee shrubs in southwestern Ethiopian moist evergreen Afromontane forest. SMF: small managed forest fragment; LMF: large managed forest; LNF: large natural forest. Bars indicate one standard deviation. Lowercase letters above bars indicate significant difference based on Tukey's hsd post hoc tests.

N1 decreased from LNF over LMF to SMF (Fig. 3.2 ; $F_{2, 37} = 38.00$ $P< 0.0001$; all pairwise comparisons $p<0.05$). We also found increasing species turnover with increasing intensity of management (beta diversity of 5.45 in LNF; 6.25 in LMF and 8.01 in SMF) (Table 3.2).

Table 3.2 Number of fragments/forest blocks and their estimated size, diversity indices and richness estimation of potential *Coffea arabica* pollinators in moist evergreen Afromontane forests of southwest Ethiopia; LNF, large natural forest; LMF: large managed forest; SMF: small managed forest.

Categories	LNF	LMF	SMF
Fragments/blocks	10	10	20
Average size of forest (ha)	> 100,000	> 100	4
Altitude ranges (m a.s.l)	1718-2108	1882-2066	1875-2080
Pollinator *beta* diversity	5.45	6.25	8.01
Average pollinator abundance per ha	1162 (63.88)	970(41.50)	844 (18.69)
Expected richness Chao2 (SD)	20.68 (3.99)	15.45(3.67)	12.86 (2.01)

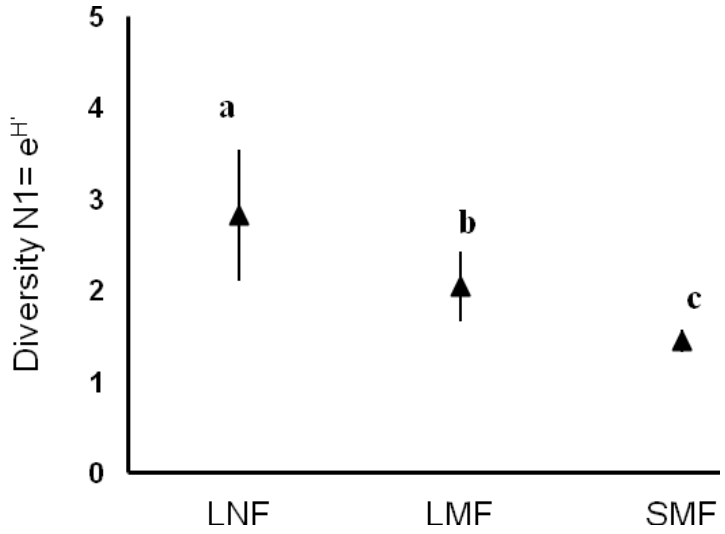


Figure 3.2 Effect of forest type on the diversity (N1) of taxonomic groups of potential coffee pollinators in southwest Ethiopian moist evergreen Afromontane forest. SMF: small managed forest fragment; LMF: large managed forest; LNF: large natural forest. Bars indicate one standard deviation.

The species accumulation curves clearly started to flatten off after few of the sampling sites (~ seven) were included in case of LNF and SMF but for LMF clear plateau was not reached (Fig. 3.3).

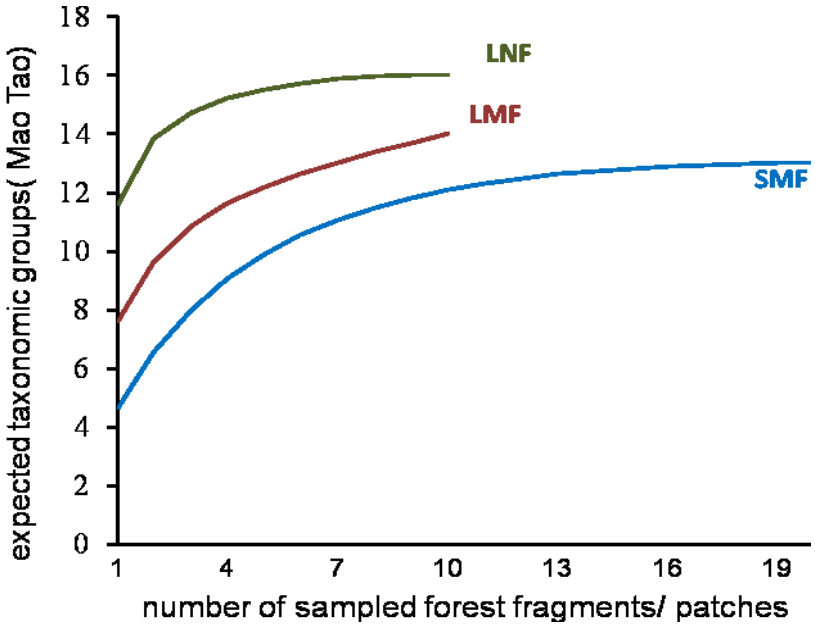


Figure 3. 3 Accumulation curves for different taxonomic groups of potential coffee pollinators against the number of sampled forest fragments/forest blocks in moist evergreen Afromontane forests of SW Ethiopia. LNF: large natural forest; LMF: large managed forest; SMF: small managed forest.

The NMS ordination (56.7% of the variance explained) showed a clear separation between the three forest management types (Fig.3.4). The MRPP anlysis showed a strong multivariate difference in species composition between the three forest management types (T= -17.68), and a more homogeneous species composition than could be expected by chance was observed within the three forest types (A= 0.295, $P <$ 0.001).

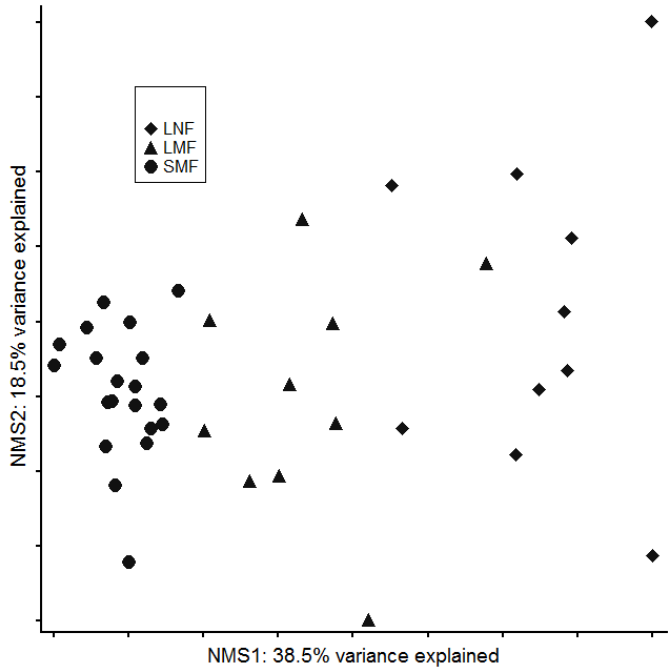


Figure 3.4 Composition of taxonomic groups of potential *C. arabica* pollinators (NMS ordination) in 40 forest fragments/forest blocks in southwest Ethiopian moist evergreen Afromontane forests. Forest blocks/fragments are labeled according to three forest types: LNF: large natural forest; LMF: large managed forest; SMF: small managed forest.

Community composition (NMS1 scores) was significantly different (P<0.001) between all forest management types (Fig. 3.5; $F_{2, 37}$ = 75.35, P< 0.0001, all pairwise comparisons: p<0.05). NMS2 scores were significantly different between LNF vs. LMF and SMF, but there was no significant difference between LMF and SMF ($F_{2,37}$ = 19.44, P< 0.0001).

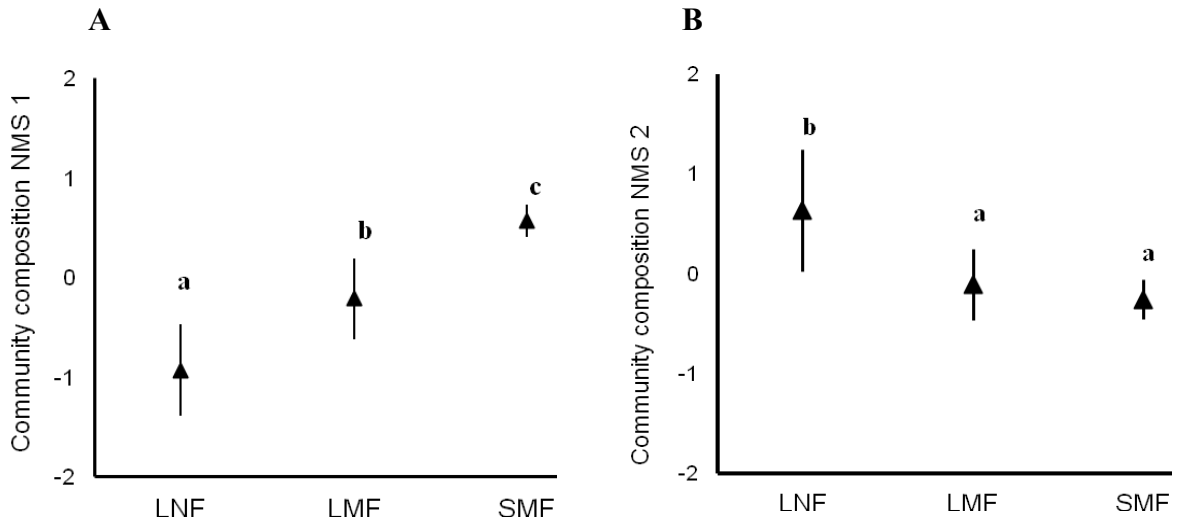


Figure 3.5 Effects of forest type on taxonomic groups composition of potential coffee pollinators in southwest Ethiopian moist evergreen Afromontane forests (NMS1 (left); NMS2 (right)). SMF: small managed forest fragment; LMF: large managed forest; LNF: large natural forest. Bars indicate one standard deviation.

3. 5 DISCUSSION

Among the identified potential pollinators of wild *C. arabica* in this study, bees, butterflies and hoverflies/syrphid flies were the most common. Honey bees, *A. mellifera* were found to be the most dominant and frequent visitors in all the sites studied, accounting for over 80% of the individuals netted on the flowering shrubs. This dominance corroborates earlier studies on coffee plant-pollinators in other tropical regions (Roubik 2002; Ricketts 2004; Vergara and Badano 2009). The dominance of honey bees in landscapes with anthropogenic impact could be attributed to two major reasons. First, honey bees are the most predominantly managed pollinators to enhance agricultural production (Potts *et al.* 2010; Winfree *et al.* 2011). Second, unlike other

pollinators, they are obligate florivores and both larvae and adult stages feed on floral products (Winfree *et al.* 2011).

Our study shows that forest management has an important impact on the diversity and species composition of the potential coffee pollinator community. Higher pollinator diversity (N1) was found in the structurally and floristically complex large natural forest as compared to intensively managed but also large coffee forest. The negative effects of forest management on pollinator diversity have been well acknowledged recently. For instance, Vergara and Badano (2009) reported higher pollinator diversity in the least managed and structurally and floristically complex rustic shaded and polyculture system, as compared to more intensively managed shaded plantations and sun coffee systems in Mexico. Similarly, Munyuli *et al.* (2010) found lower bee species diversity in intensified land use system in Uganda. The likely explanation for the decline of the diversity of pollinators along increased intensity of anthropogenic management could be related to reduced floral resources and nesting sites (Winfree *et al.* 2009), i.e. reduced taxonomic and structural diversity. Other studies also showed that floral resources are the major limiting factor for pollinators such as bees (Roulston and Goodell 2011), butterflies and moths (Ockinger and Smith 2006), and syrphid flies (Meyer *et al.* 2009). Because the managed coffee forests (LMF and SMF) under study have been structurally and floristically degraded due to human interventions for boosting coffee productivity (Senbeta and Denich 2006; Schmitt *et al.* 2009; Aerts *et al.* 2011; Hundera *et al.* 2013a), it was expected to find an impoverished insect diversity on flowering coffee shrubs. In particular the annual slashing of all plants other than coffee in the understorey may be responsible for pollinator decline, as earlier studies have shown the negative effects of removing understory flowering plant species on bee diversity (e.g. Perfecto *et al.* 1996; Jha and Vandermeer 2009). Forest canopy management also likely reduced the number of potential nesting sites for pollinators, as large old trees and dead standing trees are usually not retained as shade trees.

Contrary to the trends for other insect groups, we found the relative abundance of honey bees to increase significantly with increasing management intensity. Our data suggests that honey bees are almost the sole visitors of coffee flowers in highly

managed SFC systems (90% of the abundance of insects on coffee flowers). Declining wild and forest dwelling pollinators due to competition for scarce resources (Winfree *et al.* 2011), differences in tolerance level to anthropogenic disturbances (Winfree *et al.* 2009), and introduction of bee hives by farmers might explain the increase of honey bee abundance in highly managed forest. The establishment of traditional bee hives on large remnant trees in the agricultural matrix and in the shade trees within the SFC forests is common practice throughout the region. Although honey bees have been reported to assure pollination in disturbed habitats (Dick 2001), they have also proven to be rather poor pollinators compared to native or wild pollinators (Aizen and Feinsinger 1994; Garibaldi *et al.* 2013). In the face of diverse threats to pollinators across the globe, reliance on a single species for pollination is also precarious (Ricketts et al. 2008).

Our study also indicated significant impacts of forest fragmentation on diversity and community composition of *C. arabica* visiting insects. LMF was indeed significantly different from SMF in terms of N1 and community composition, although both forest types had the same management intensity (both SFC systems, Aerts *et al.* 2011). Different mechanisms may explain these habitat fragmentation effects on the diversity of coffee flower visiting insects and their community composition of managed montane coffee forests. One mechanism could be the degradation of habitat quality due to edge effects (Broadbent *et al.* 2008). Since the edge effect is much higher and unavoidable in small fragments, the microclimate in terms of relative humidity, air temperature, wind speed within the fragmented habitat will be altered (Didham and Lawton 1999; Broadbent *et al.* 2008). Because different pollinator taxa are expected to have different responses to such changed microenvironments, the abundance of those pollinators that are not capable of tolerating the altered habitat will gradually decline and /or become extinct if the change persists (Potts *et al.* 2010). Also decreased forest fragment area and increased spatial isolation may negatively affect pollinator abundance and diversity (Kearns *et al.* 1998; Boreux *et al.* 2013). Forests may have become too small to sustain pollinator communities, e.g. through limited resource availability or genetic and

demographic effects (Winfree *et al.* 2009), or too isolated to attract a large diversity of pollinators (Steffan-Dewenter and Tscharntke 1999; Garibaldi *et al.* 2011).

3. 6 CONCLUSIONS

Our study showed that both forest management and fragmentation have negative effects on potential *C. arabica* pollinator abundance and diversity. We also showed a significant shift in the flower visiting insect community towards honey bee dominated communities with increasing management intensity. Although the introduction of bee hives can contribute to coffee productivity and to improved livelihoods through farm product diversification, it is likely unable to provide a resilient pollination service (Ricketts *et al.* 2008; Garibaldi *et al.* 2013). This may negatively affect coffee productivity in the long term as pollination services by managed honey bees are expected to decline under current climate change scenarios (Rader *et al.* 2013). Therefore, introduction of bee hives in fragmented coffee forests should be complementary with other practices that conserve and promote wild pollinator diversity, including the retention of trees with nesting cavities or alternative pollen and nectar sources.

CHAPTER 4:

EFFECTS OF FOREST MANAGEMENT ON MATING PATTERNS, POLLEN FLOW AND INTERGENERATIONAL TRANSFER OF GENETIC DIVERSITY IN WILD ARABICA COFFEE (*COFFEA ARABICA* L.) FROM AFROMONTANE RAINFORESTS

This chapter is adapted from:

Berecha G, Aerts R, Vandepitte K, Van Glabeke S, Roldan-Ruiz I, Muys B and Honnay, O (2014) Effects of forest management on mating patterns, pollen flow and intergenerational transfer of genetic diversity in wild Arabica coffee (*Coffea arabica* L.) from Afromontane rainforests. *Biological Journal of the Linnean Society* ***112****: 76-88.*

4. 1 SUMMARY

Coffea arabica, the wild ancestor of all commercial Arabica coffee cultivars worldwide, is endemic to the montane rainforests of Ethiopia. These forests, which harbour the most important *C. arabica* gene pool, are threatened by increasing anthropogenic disturbance, potentially altering the mating patterns, pollen dispersal and maintenance of genetic diversity in *C. arabica* understory populations. We genotyped 376 adult coffee shrubs and 418 progenies from three natural unmanaged, and three highly managed coffee populations, using 24 microsatellite markers. Mating system analysis of *C. arabica* yielded an overall multilocus outcrossing rate of 76%, which contrasts the common knowledge that *C. arabica* is a predominantly selfing species. In highly managed coffee populations, paternity could be assigned to 78% of the progenies, whereas in the unmanaged natural coffee populations, only 57% of the progenies could be assigned to a father, indicating reduced long distance pollen dispersal in managed forests. Furthermore, the fraction of selfed progenies was significantly higher in managed (23%) compared to unmanaged (10%) coffee forests. Finally, the lack of spatial genetic structure in all studied populations suggests high seed dispersal in unmanaged populations, and intense berry harvesting and coffee planting in the managed populations. Our results imply that *in situ* conservation of the wild gene pool of *C. arabica* must focus on limiting intensification of coffee forest management, as decreased pollen dispersal and increased selfing in *C. arabica* in intensively managed populations may increase the risk of genetic erosion.

Keywords: Afromontane rainforest, coffee, crop wild relative, gene flow, habitat degradation, mating system

4. 2 INTRODUCTION

Coffee is one of the largest export commodities and is a livelihood crop for over 100 million people worldwide (Vega 2008). Of the 124 coffee species described to date (Davis *et al.* 2006; 2010; 2011), the commercial coffee production relies on only two species, *Coffea arabica* (Arabica or highland coffee, ca. 70%) and *C. canephora* (robusta or lowland coffee, ca. 30%) (ICO 2013). Currently, over 60 countries throughout the tropics grow coffee, on more than 11 million ha (Waller *et al.* 2007). Despite the current wide geographical range of Arabica coffee cultivation, the genetic base of the used cultivars is narrow (Anthony *et al.* 2002). Whereas this has resulted in a crop with homogenous agronomic behaviour (Lashermes *et al.* 2009), adaptability in response to environmental changes and climatic hazards is expected to be low.

The ancestor of cultivated Arabica coffee is wild *C. arabica* L., a shrub endemic to southwest Ethiopia (Anthony *et al.* 2001; 2002), where it is naturally occurring in the understory of the Afromontane moist forests. Wild populations of Arabica coffee in these rainforests are genetically diverse, and likely possess desirable traits that can be used to improve the cultivated varieties of *C. arabica* worldwide (Aga *et al.* 2005; Tesfaye *et al.* 2013; Chapter 2). At least within Ethiopia, active selection and hybridization activities with wild coffee individuals over the last decades have led to numerous landraces or farmers' varieties (reviewed in Labouisse *et al.* 2008). The importance of the Ethiopian wild coffee populations can be expected to increase in the future as breeders attempt to address the threats of the combination of global environmental change and a higher demand for food (Foley *et al.* 2011). The conservation of the genetic diversity of *C. arabica* in Ethiopian rainforests is therefore of major importance, but the conservation of wild gene pools of cultivated species generally remains an often undervalued challenge for conservation biologists (Honnay *et al.* 2012).

The traditional coffee production systems practiced in Ethiopia are Forest Coffee (FC) and Semi-Forest Coffee (SFC), and they differ profoundly by the intensity of the rainforest management (Senbeta and Denich 2006; Labouisse *et al.* 2008; Aerts *et al.*

2011). While forests under the FC system experience little or no disturbance, interventions such as removal of shrubs and emerging seedlings, and selective thinning of the upper canopy to increase coffee yield, are typical for the SFC system (Schmitt *et al.* 2009; Aerts *et al.* 2011). Because yield of coffee shrubs in FC systems is very low (15kg ha^{-1} yr^{-1}; Schmitt *et al.* 2009), increasing human population densities and increasing demand for Arabica coffee on the world market have caused a major shift from FC systems towards the more intensively managed SFC systems (Senbeta and Denich 2006). Not much is known so far, however, about how intensified rainforest management may affect the *in situ* conservation of the wild *C. arabica* gene pool (but see Chapter 2).

As forest management practices typical for the SFC system have important consequences for forest structure and plant species composition (Senbeta and Denich 2006; Schmitt *et al.* 2009), they can also be expected to strongly affect pollinator abundance and behavior (Brosi *et al.* 2008; Brosi 2009; González-Varo *et al.* 2009; Jha and Vandermeer 2010), and therefore to affect the mating patterns (i.e. the pattern of pairing of gametes and their genetic relatedness) in the understory coffee populations (Obayashi *et al.* 2002; Eckert *et al.* 2010). A recent meta–analysis of 22 studies involving 27 plant species indeed showed significantly lower outcrossing rates in plant species in disturbed compared to undisturbed habitats (Eckert *et al.* 2010). At the population level, the alteration of mating patterns can have significant consequences with respect to the intergenerational transfer of genetic diversity. As severe anthropogenic disturbance may decrease pollinator availability, this may result in reduced pollen dispersal and increased selfing, decreasing the effective population size, and reducing the genetic diversity of the progeny produced (Tani *et al.* 2009; Fuchs and Hamrick 2011; Zalucki *et al.* 2013). Increased selfing or mating between genetically related individuals can also be expected to result in a strong local pedigree structure, with higher genetic similarity among neighboring than among more distant individuals (Vekemans and Hardy 2004). Quantifying small scale population genetic structure can therefore yield insight in realized mating in the adult generation (Krauss *et al.* 2009; Zhao *et al.* 2009; Barluenga *et al.* 2011).

The mating system of coffee under natural conditions, and its reliance on pollinator services for fruit set, can also be expected to mediate the impact of forest management on mating patterns. Owing to its reported self-compatibility and high degree of autogamous selfing (Free 1993), fruit set in Arabica coffee has been traditionally considered to be relatively independent from insect pollinators. However, empirical studies have recently shown a considerable increase in fruit set of highland coffee in pollinator rich environments (Roubik 2002; Klein *et al.* 2003; Veddeler *et al.* 2008; Vergara and Badano 2009). So far, a formal analysis of the mating system of *C. arabica* in its natural habitat, based on genetic markers, is lacking.

The general objective of this study was to quantify mating patterns in wild *C. arabica* populations in their natural habitat, the Ethiopian montane rainforests. To that end, we used 24 Simple Sequence Repeat (SSR) markers to genotype 376 adult coffee shrubs and 418 progeny plants from three FC populations and three SFC populations. Our first specific aim was to examine the mating system of wild *C. arabica*. Our second specific aim was to compare (i) selfing and outcrossing rates; (ii) pollen flow patterns; (iii) intergenerational transfer of genetic diversity; and (iv) spatial genetic structure, between coffee populations from natural FC systems, and coffee populations from highly managed SFC systems.

4. 3 MATERIALS AND METHODS

Study species

Highland coffee (*Coffea arabica* L.: Rubiaceae) is the only self-fertile species of the *Coffea* genus (Davis *et al.* 2006; 2010). *C. arabica* is an allotetraploid (2n=4x=44), resulting from a relatively recent natural hybridization between its two putative diploid parents, i.e. *C. canephora* and *C. eugenoides* (Lashermes *et al.* 1999; Maurin *et al.* 2007). *C. arabica* flowers and bears fruit at an age of three years (Free 1993; Puff 2003). In SW Ethiopia, flowering is triggered by rain showers after a dry spell of 3–4 months, often between January and April (Chapter 2). The stigma is receptive when a

flower opens at dawn and the anthers dehisce soon afterwards (Klein *et al.* 2003). The base of the style secretes nectar which attracts different pollinator taxa, in Ethiopia mainly bees (Fichtl and Admasu 1994).

Sample collection and study population

Coffee leaf samples were collected from six populations in the Afromontane rainforest of the Jimma zone in the Oromia region in SW Ethiopia (Fig.4.1). Three populations were located in the remote part of the Belete-Gera National Forest Priority Area (NFPA). The canopy of this natural forest consists of a mixture of broad-leaved tree species 10–30m tall with emergent trees that can reach a height of 30-40m. Dominant tree species include *Syzygium guineense, Prunus africana, Olea welwitschii, Schefflera abyssinica* and *Ilex mitis.* These sites experienced no or very little forest management, and are further referred to as FC systems. The other three populations were located in three intensively managed coffee forests that have been continuously managed for coffee production over the past 50 years. These fragments are located in the coffee producing agricultural landscape east of NFPA. These forests experienced rigorous tree thinning and removal of understory shrubs (Aerts *et al.* 2011). The dominant tree species here include *Albizia gummifera, A. schimperiana, Croton macrostachyus* and *Millettia ferruginea*. We further refer to these sites as SFC systems.

We established rectangular sampling plots at each study site with average plot size of 400 m^2, aiming to include approximately 60 adult coffee shrubs (Senbeta and Denich 2006). All coffee shrubs (n = 376) were sampled and leaves were dried on silica gel. While sampling, we randomly marked five fecund shrubs per plot for later berry collection. Fifty to sixty open pollinated berries were collected from each marked shrub and progenies were raised in a greenhouse. After germination, leaf samples were randomly collected from on average 14 (min 9; max 15) progenies per mother shrub (total 418 samples), and also dried on silica gel.

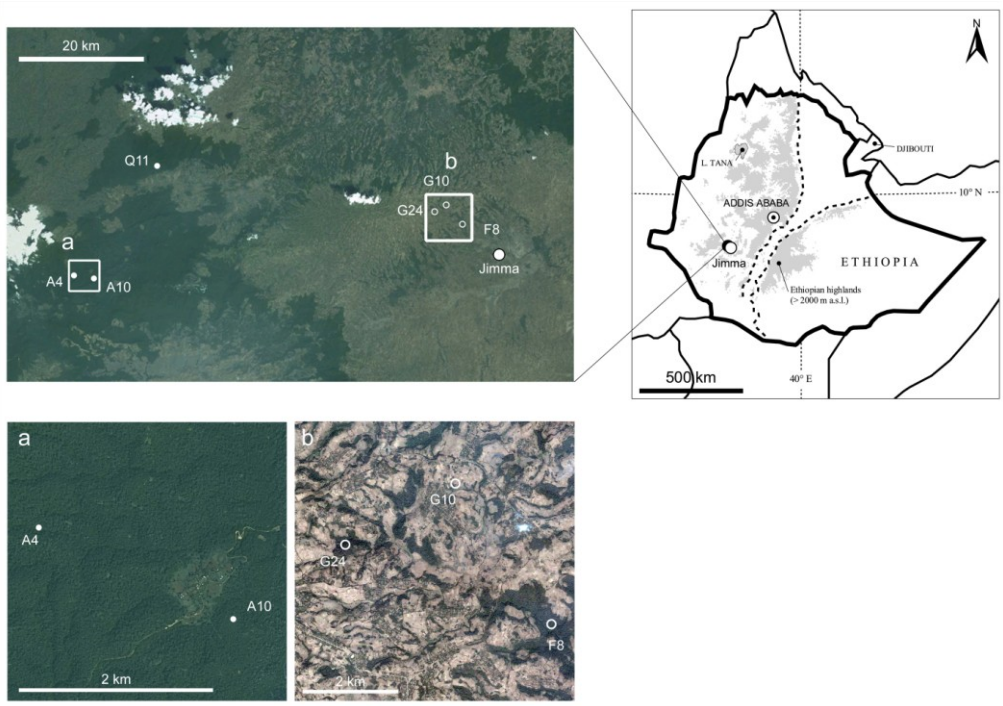


Figure 4.1 Map showing sampling plots in the Afromontane forests of southwest Ethiopia, Letters represent sampled forest fragments: a, unmanaged continuous forest; b, intensively managed forest fragments, A4, A10 and Q11 refer to the studied populations in FC whereas F8, G10 and G24 refer to the populations in SFC; Satellite imagery © 2013 DigitalGlobe GeoEye and Cnes/Spot Image via Google Earth.

Genomic DNA extraction and SSR genotyping

Prior to DNA extraction, leaf material was homogenized with a mill (Mixel Mill MM, 200, Retsch®, Haan, Germany). Genomic DNA was extracted from 20-22mg of dried leaf samples using the CTAB protocol of the NucleoSpin® Plant II isolation kit (Machery-Nagel, Duren, Germany). Compared to the standard protocol, we increased incubation time during cell lysis to 60 minutes at 65°C and used a two-step elution procedure incubated at 70°C for optimal recovery of bound nucleic acids. DNA samples were assayed using the 24 microsatellite loci (from Combes *et al.* 2000; Silvestrini *et al.* 2007; Hendre *et al.* 2008; López-Gartner *et al.* 2009) detailed in Chapter 2 above. The 24 SSRs were amplified in six multiplex PCRs using a Gene Amp® PCR system 9700 thermal cycler (Applied Biosystems®, CA, USA). PCR reactions were performed in total

sample volumes of 10µL, consisting of 5µL Qiagen®Multiplex PCR mix, 0.2µl of each forward and reverse primer of one mix (10µM) complemented with RNase-free MQ water and 2µL sample / template DNA. The multiplexes had equal thermocycling profiles with 15 min initial denaturation at 95°C, followed by 25 cycles of denaturing at 94°C for 30sec, annealing at 57°C for 90 sec and extension at 72°C for 1min with a final extension step at 60°C for 30min. Then, 1µL of the PCR reaction was added to a solution of 8.8µL formamide and 0.2µL of the Applied Biosystems GeneScan^{TM} 500 LIZ® size standard. Sized fragments were scored using GeneMapper®v 4.0 (Applied Biosystems).

Despite their extensive use as markers of choice in many genetic studies dealing with diploid species, the application of microsatellite markers (SSRs) in a polyploid species is limited by the difficulty to identify true genotypes of partial heterozgotes. Here, we used ATetra (Van Puyvelde *et al.* 2010) to handle codominant microsatellite data of allotetraploid species. ATetra computes suits of diversity measures (see below). However, similar techniques do not exist for paternity, mating system and spatial autocorrelation analysis in polyploid species. For these analyses, we transformed our codominant microsatellite data into a dominant data set, similar to an amplified fragment length polymorphism (AFLP) profile, by considering each microsatellite allele as an independent locus. Although an inherent loss of information (heterozygosity) is expected in this kind of transformation, the approach has been widely used by several studies (e.g. Vanderpoorten *et al.* 2011; Sampson and Byrne 2012; Vallejo-Marin and Lye 2013).

Paternity analysis

Parent *versus* offspring reconstruction was performed in the six studied populations, using FaMoz (Gerber *et al.* 2000). We used a likelihood approach to detect the most likely father using the logarithm of likelihood ratio (LOD score) for dominant markers. Paternity assignment was carried out after setting LOD thresholds using the cross-section of the LOD distributions of simulated offspring from inside *versus* outside stands assuming an error rate of 1% and 0.01% for calculation and simulation, respectively and

a deviation from HWE of 0.25 based on our outcrossing estimation. Offspring were assigned to the most likely parent when the LOD score of that parent was higher than the LOD threshold. When the parent–offspring LOD score fell below the LOD threshold, offspring paternity was left unassigned. Once paternity assignment was completed, cryptic gene flow was estimated using 10,000 simulations. Because cryptic gene flow was zero for two populations and near zero for the other populations, all unassigned offspring was considered to result from pollen inflow. Next, to investigate the impact of management (FC vs. SFC) on pollination patterns, we used binomial Generalized Linear Models for event/trial data. Dependent variables were the number of selfed offspring and the number of offspring sired by outside sources, relative to the number of genotyped offspring per mother plant. Management type and population nested within management type were included as independent factors. Analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL).

Transfer of genetic diversity between generations

The within population genetic diversity of each population and each generation (adult trees *versus* seedlings germinated from the berries) were estimated using the program ATetra 1.2a (Van Puyvelde *et al.* 2010). We calculated expected heterozygosity corrected for sample size (HEc); Shannon–Wiener Diversity Index corrected for sample size (H'c); Nei's measure of population differentiation (G_{ST}), the interpopulational gene diversity (D_m), and Nei's genetic distance (D). We used a Mann-Whitney test to compare average percentage intergenerational transfer of genetic diversity between FC and SFC systems.

Spatial genetic structure

Estimation of spatial genetic structure (SGS) helps to gain insights into the dispersal behavior of genes (pollen and seed) within populations. We used GENALEx 6.5 (Peakall and Smouse 2006; 2012) to assess fine scale spatial genetic structure of all adult shrubs within each management system using genetic and spatial data. The software (GENALEx) follows the multivariate approach described in Smouse and Peakall (1999), and tests for significance by establishing lower and upper 95 %

boundaries for the autocorrelation coefficient(*r*) based on 999 random simulations of the null model *i.e.* no autocorrelation. Similar confidence intervals are established around the estimate of *r* using 999 bootstraps, and structure is inferred for non-overlapping positive distance classes.

Mating system analysis of forest coffee

To gain insight in the mating system of *C. arabica*, overall mating system parameters were quantified in both the three FC populations and the three SFC populations. To estimate outcrossing rates, we employed the multi-locus method of moments procedure which is a weighted average of individuals within families, using population alleles frequencies (Ritland 2002). First, we estimated outcrossing rates following the Newton-Raphson method for 24 loci, with standard errors based on 1000 bootstraps, by including known maternal genotypes. Second, we estimated the parameters of the correlated matings model, using the maximum-likelihood approach. For each of the six populations, we estimated: (i) multilocus outcrossing rates (t_m), (ii) biparental inbreeding or the difference between the multilocus and single locus outcrossing rate (t_m-t_s), (iii) correlation of paternity (r_p), and (iv) the correlation of selfing among family (r_s).

4. 4 RESULTS

Paternity assignment

The theoretical cumulative exclusion probability calculated over 24 loci was high. The mean single parent exclusion probability was 0.971 and the mean parent pair exclusion probability was 0.998 (Table 4.1).

Table 4.1 Paternity assignment in six populations of Afromontane coffee forests using 24 SSR markers, A4, A10 and Q11 refer to the studied populations in FC whereas F8, G10 and G24 refer to the populations in SFC.

Population	N	Ex. Prob.	Kw	Ka	Pw	S	O	CYG
Forest coffee (FC)								
Afalo-4 (A4)	65	96.73	29.00	37.00	56.92	3.00	56.00	0.000
Afalo-10 (A10)	59	93.86	22.00	38.00	64.40	0.00	65.00	0.015
Qacho-11(Q11)	69	98.32	35.00	34.00	49.27	26.00	28.00	0.000
Mean		**96.30**	**28.67**	**36.33**	**56.87**	**9.67**	**49.70**	**0.005**
Semi-forest coffee (SFC)								
Fetche-8 (F8)	75	97.22	25.00	50.00	66.67	33.00	33.00	0.004
Garuke-10 (G10)	75	98.64	17.00	58.00	77.33	8.00	71.00	0.004
Garule-24 (G24)	75	97.96	7.00	68.00	90.67	28.00	61.00	0.011
Mean		**97.94**	**16.33**	**58.67**	**78.22**	**23.00**	**55.00**	**0.006**

N number of progenies genotyped; **Kw** number of progenies not assigned from the study population; **Ka** number of progenies assigned from within study plot; **Pw** per cent progenies sired by a single father from within the study plot; **S** selfing rate at individual maternal shrub level; **O** outcrossing rate at individual maternal shrub level; **CYG** cryptic gene flow.

Of the 418 genotyped progenies, 78.2% (SE 6.96) and 56.8% (SE 4.37) were unambiguously assigned to a single father from within the study plots in SFC and FC populations, respectively. Of the 225 genotyped SFC offspring, 53 (23.0%, SE 0.065) were the result of selfing, while 18 (9.7%, SE 0.050) of the 193 genotyped FC offspring were self-pollinations (Fig. 4.2). GLMs confirmed that the fraction of selfed offspring (*S*)

and the fraction of offspring sired by outside sources (P_{in}) significantly differed between management systems (*S*: $\chi^2 = 247.17$, $P<0.001$, P_{in}: $\chi^2 = 57.07$, $P<0.001$).

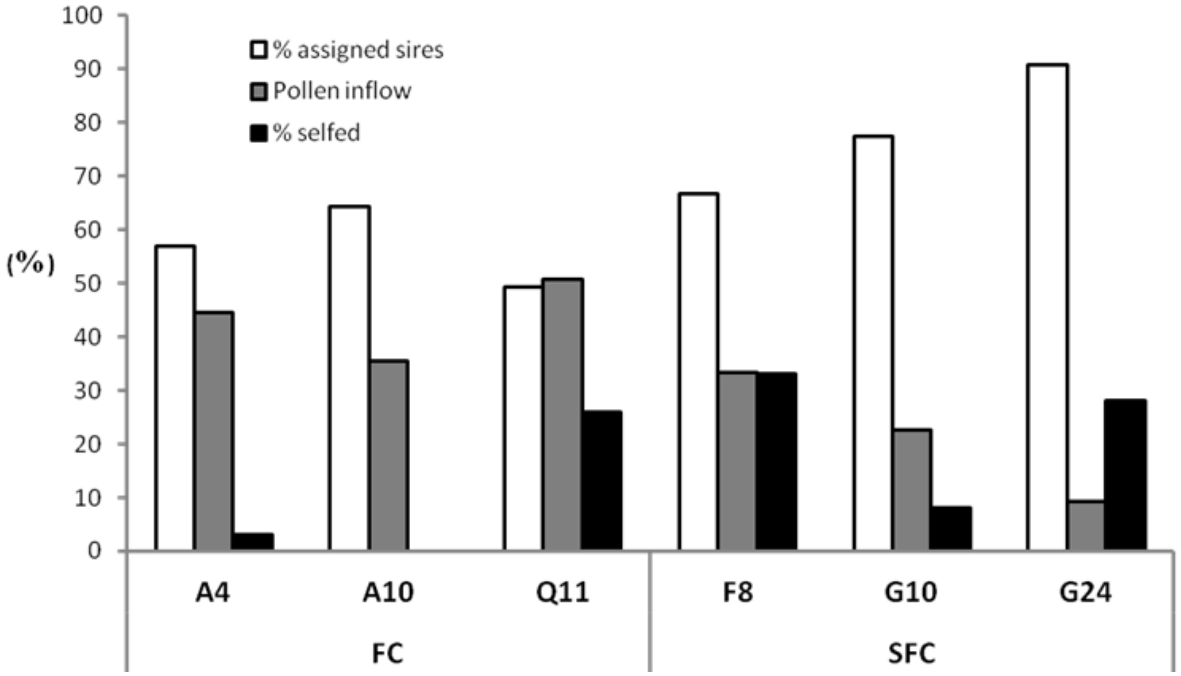


Figure 4.2 Percentage of progenies assigned to fathers from within the study plot (white bars) percentage of pollen inflow from outside study plot (gray bars) and fraction of selfed progenies (black bars) in the six studied populations: FC unmanaged forest coffee; SFC intensively managed semi-forest coffee, A4, A10 and Q11 refer to the studied populations in FC whereas F8, G10 and G24 refer to the populations in SFC.

Intergenerational transfer of genetic diversity and spatial autocorrelation

Transfer of genetic variation from the adult generation to the progeny tended to be higher in SFC, compared to FC populations (Fig. 4.3 and Table 4.2). Expected heterozygosity corrected for sample size (HEc) was reduced by on average 5.29% in the FC populations, compared to 2.0% in SFC populations (Fig. 4.3a). Similarly, we found a higher reduction of the genetic diversity (Shannon–Wiener Diversity Index

corrected for sample size (H'c) in FC, compared to SFC (mean = 14.39, SE 0.04 vs. 9.48, SE 0.05, respectively) (Fig. 4.3b). None of these differences in loss of genetic diversity between FC and SFC were significant, however, according to Mann Whitney tests.

Table 4.2 Estimates of expected heterozygosity corrected for sample size ($H_{E,C)}$ Shannon–Wiener Diversity Index corrected for sample size (H'c); Nei's measure of population differentiation (G_{ST}) the interpopulational gene diversity (D_m) and Nei's genetic distance (D) for *Coffea arabica* populations in SW Ethiopia based on 24 microsatellite loci, A4, A10 and Q11 refer to the studied populations in FC whereas F8, G10 and G24 refer to the populations in SFC.

Population	**HEc**		**H' c**		**Adult vs. progeny subpopulations**		
	Adult	Progeny	Adult	Progeny	G_{ST}	D_m	D
Forest coffee							
Afalo(A4)	0.5692	0.5349	0.9798	0.8676	0.0034	0.0039	0.0526
Afalo(A10)	0.5208	0.4376	0.9003	0.6611	0.0057	0.0067	0.0306
Qacho(Q11)	0.6011	0.5600	1.0389	0.9586	0.0098	0.0134	0.0629
Mean	**0.5637**	**0.5108**	**0.9730**	**0.8291**	**0.0063**	**0.0080**	**0.0487**
Semi-forest coffee							
Fetche(F8)	0.5578	0.5142	0.9741	0.8764	0.0174	0.0228	0.0465
Garuke(G10)	0.5731	0.5416	1.1056	0.9259	0.0155	0.0205	0.0448
Garuke(G24)	0.5314	0.5467	0.9665	0.9597	0.0399	0.0593	0.0912
Mean	**0.5541**	**0.5342**	**1.0154**	**0.9206**	**0.0243**	**0.0342**	**0.0608**

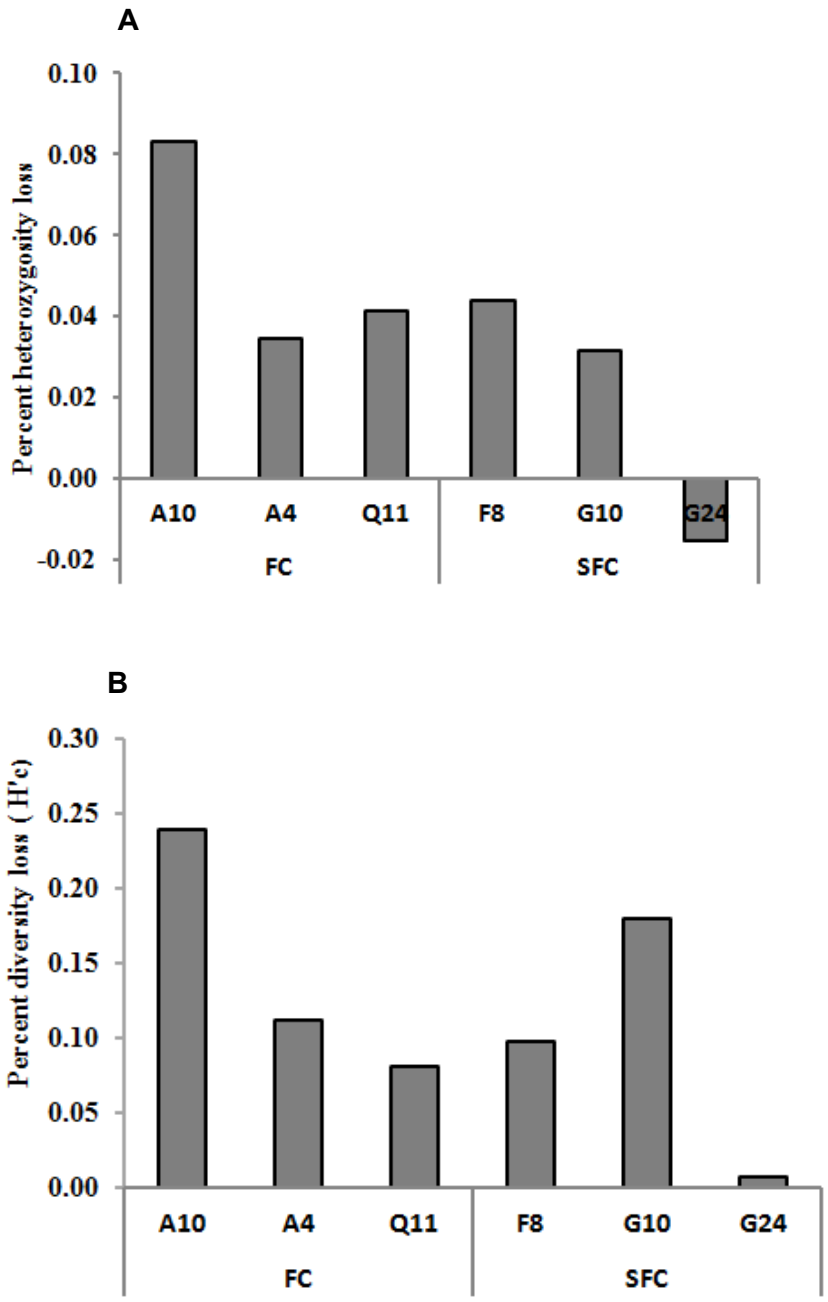


Figure 4.3 Relative heterozygosity losses (A) and reduction in genetic diversity (H'c) (B) from the parental to the offspring generation for unmanaged forest coffee (FC) and intensively managed semi-forest coffee (SFC), A4, A10 and Q11 refer to the studied populations in FC whereas F8, G10 and G24 refer to the populations in SFC. The mean expected heterozygosity loss was 0.053 (SE 0.015) in FC and 0.020 (SE 0.018) in SFC while the mean genetic diversity (H'c) loss was 14.39 (SE 0.048) in FC and 9.48 (SE 0.049) in SFC.

To visualize the dispersal behavior of genes (pollen and seeds) within the population, the spatial autocorrelation analysis was performed for the adult population. Accordingly, we found non-significant fine scale spatial autocorrelation in the studied adult populations (see Fig.4. 4 for some examples).

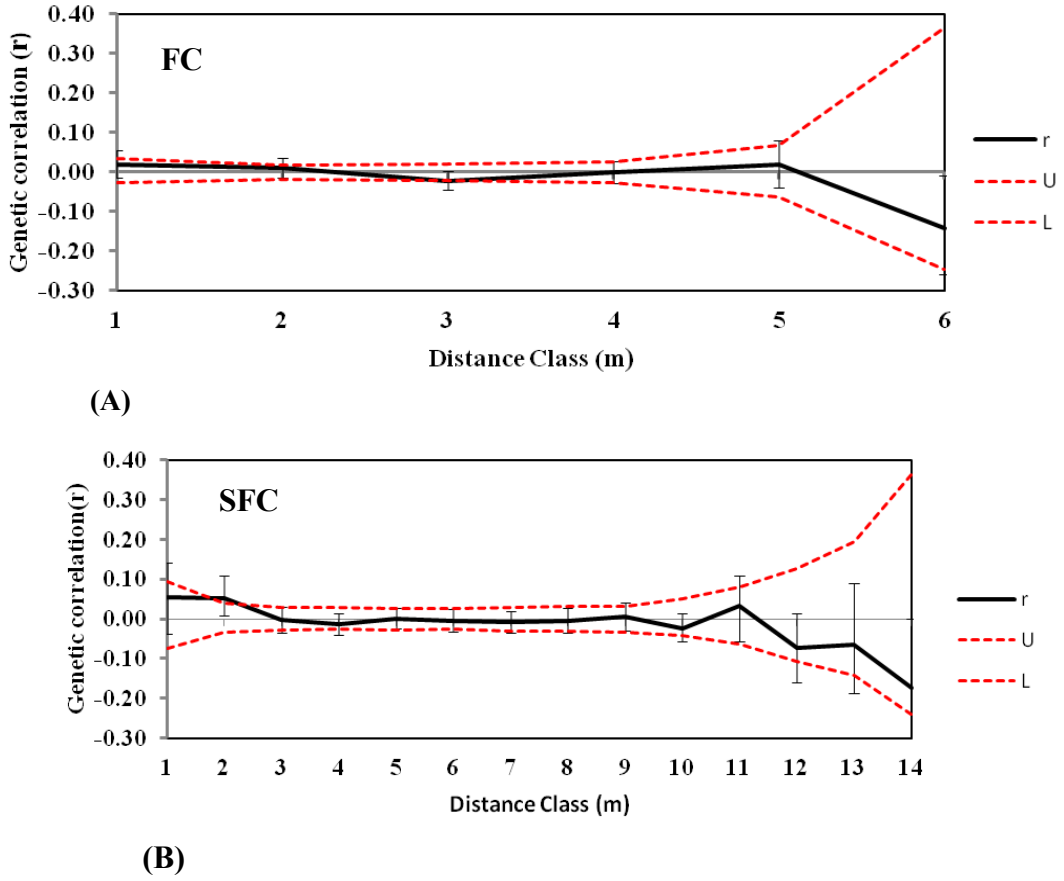


Figure 4.4 Spatial auto-correlation for adult populations of *C. arabica* in Afromontane coffee forest, SW Ethiopia. Letters represent studied populations: **A**, least managed coffee forest populations; **B**, intensively managed semi-forest coffee populations. The dashed lines represent upper and lower 95% confidence intervals (10,000 permutations).

Mating system in *Coffea arabica*

The mean multilocus outcrossing rate (t_m) for *C. arabica* in the FC populations was 0.76 (SE 0.093). The mean biparental inbreeding was 0.155 (SE 0.030) while the mean

correlation of selfing among families was 0.814 (SE 0.100). We found a mean correlated paternity of 0.200 (SE 0.117) (Table 4.3). Our mating analysis for the intensively managed fragmented coffee forests (SFC) yielded a mean t_m of 0.70 (SE 0.038) (Table 4.3). The mean correlated paternity, correlation of selfing among families and biparental inbreeding in SFC populations were 0.265 (SE 0.051), 0.885 (SE 0.066) and 0.074 (SE 0.044), respectively.

Table 4.3 Location and results of a mating system analysis of wild *Coffea arabica* in SW Ethiopia using 24 SSR markers, A4, A10 and Q11 refer to the studied populations in FC whereas F8, G10 and G24 refer to the populations in SFC.

Stand	Lat(N)	Long(E)	elve(m)	t_m	t_s	t_m-t_s	Rp	Rs
Forest coffee (FC)								
Afalo-4 (A4)	7.6395	36.2067	1987	0.736	0.557	0.179	0.167	0.787
Afalo-10 (A10)	7.6307	36.2241	1825	0.932	0.835	0.096	0.015	0.657
Qacho-11 (Q11)	7.7868	36.3238	2108	0.612	0.422	0.191	0.417	0.999
Semi-forest coffee (SFC)								
Fetche-8 (F8)	7.7106	36.7617	1908	0.663	0.673	-0.010	0.175	0.999
Garuke-10 (G10)	7.7368	36.7420	2025	0.663	0.570	0.094	0.350	0.886
Garuke-24 (G24)	7.7256	36.7227	2062	0.776	0.637	0.139	0.269	0.771

t_m multilocus outcrossing rate; **t_s** single locus out crossing rate; **t_m-t_s** biparental inbreeding; **Rp** correlation of paternity; **Rs** correlation of selfing among families.

4. 5 DISCUSSION

Mating system in wild *Coffea arabica*

C. arabica is widely perceived as a predominantly selfing species with an outcrossing rate of ca. 10% (e.g. Free 1993; Anthony *et al.* 2001; Davis *et al.* 2006). This knowledge stems from pollination studies in *C. arabica* cultivars, reporting outcrossing rates of less than 10% in Colombia (Castillo-Zapata 1976), 12% in Brazil (Carvalho and Krug 1949), and 7-15% in Kenya (Van der Vossen 1974). Our mating system analysis, which was the first to examine, mating patterns in wild Arabica coffee populations based on the inheritance of genetic marker variation, yielded completely different values. We found an overall multilocus outcrossing rate as high as 76%, typical for mixed mating species (Goodwillie *et al.* 2005; Busch *et al.* 2010; Zhu and Lou 2010). This supports the prediction of Meyer (1965), who has reported self fertility of 40 to 60% in wild Arabica coffee shrubs that were collected from the wild and planted at a common garden in Jimma, Ethiopia, but who has suggested higher outcrossing rates in wild Arabica coffee populations. Throughout the domestication process of *C. arabica*, self-pollination and biparental inbreeding, due to low pollinator availability and the presence of genetically similar genotypes, respectively, may have increasingly promoted self-fertility. If seed set following self-fertilization is low due to expression of lethal alleles in the homozygous state, then successive generations of inbreeding can increase self-fertility due to the selective decrease in frequency of deleterious alleles (Johnston and Schoen 1996; Crnokrak and Barrett 2002). The abundant availability of pollinators, pollination by multiple pollen donors (as indicated by the moderate correlated paternity value (0.2), and relatively low biparental inbreeding (0.155), all likely maintain high outcrossing rates in wild Arabica coffee populations. Pollination by multiple pollen donors and relatively low biparental inbreeding are also in accordance with the typical low density of plants in wild Arabica coffee populations (Senbeta and Denich 2006; Labouisse *et al.* 2008; Schmitt *et al.* 2009; Aerts *et al.* 2011), promoting longer flight distances. Moreover, substantial gene flow between populations of wild Arabica coffee in Ethiopian has been reported (Aga *et al.* 2003; Tesfaye *et al.* 2013).

Effect of forest management on pollen dispersal

Paternity assignment demonstrated modest but significant effects of coffee forest management on mating patterns. Although outcrossing was considerable in both systems, we found a significantly higher fraction of selfed progenies in SFC populations (n = 53; 23.0%) compared to FC populations (n =18; 9.7%). Also the fraction of outcrossed progenies sired by outside sources was significantly higher in FC populations (n = 86; 41.0%) compared to SFC populations (n = 49; 22.0%), suggesting more intensive pollen inflow and longer pollen dispersal distances in FC systems. Increased pollen inflow rates and lower selfing in FC compared to SFC populations may be attributed to altered foraging behavior of pollinators in response to differences in floral display size and in abundance of flowering coffee shrubs between the two management systems. The density of flowering coffee shrubs is higher in SFC than FC systems (Senbeta and Denich 2006; Schmitt *et al.* 2009; Aerts *et al.* 2011), and the rigorous canopy thinning practices in SFC systems encourage the development of more fruiting branches per tree, increasing the number of flowers produced per shrub. Large floral displays can be expected to tempt pollinators to spend more time on one shrub (Brunet and Sweet 2006; Williams 2007). Because pollinators visiting hermaphroditic flowers often deposit mixed pollen of different individuals, including self pollen (Karron *et al.* 2006), this altered pollinator behavior in response to higher floral densities is expected to promote selfing (geitonogamy) in self-compatible, animal pollinated species (Kalisz *et al.* 2007), as seems supported by the higher selfing rate within SFC compared to FC populations.

The effects of differences in flower densities on mating patterns between management types may further have been exacerbated through reduction of the pollinator diversity in the SFC systems. Anthropogenic disturbances such as forest management may cause changes in microclimate and in availability of nesting sites for insect pollinators (Klein *et al.* 2008), thus negatively affecting the diversity of the pollinator communities (Kremen *et al.* 2002; Brosi *et al.* 2008; Klein 2009), and the frequency of plant-pollinator interactions (Steffan-Dewenter and Westphal 2008; Eckert

et al. 2010). As a consequence, mating patterns and gene dispersal may become jeopardized (Brys and Jacquemyn 2012; Mannouris and Byers 2013).

Consequences of forest management on intergenerational genetic diversity

The transmission rate of genetic variation from the adult to the progeny generation was not significantly different in FC compared to SFC populations. Although the demonstrated loss of genetic variation does not indicate that genetic variability is lost throughout generations, as we only sampled the progeny of 5 mother shrubs, representing only one generation, they do suggest that forest management is not negatively impacting the transfer of genetic variation. One possible explanation for this is the introduction, through planting, of coffee shrubs by local farmers in SFC systems, artificially increasing genetic variation. Our result in Chapter 2 has indeed showed high genetic variation in SFC populations attributable to anthropogenic introduction of diverse genotypes.

Effect of forest management on spatial genetic structure

Fine-scale spatial genetic structure was non-significant in all six studied populations. These findings corroborate the unexpectedly high outcrossing rate in wild coffee populations. A likely explanation for the absence of spatial genetic structure in the FC populations is the high number of seed dispersal events by different vectors such as birds, monkeys and bats (Senbeta and Denich 2006), and very low seedling recruitment, due to intensive competition between coffee and other plant species on the forest floor. In SFC populations, the combination of high seed removal (through coffee harvesting) and the introduction of coffee genotypes from other sites may have contributed to this pattern (Schmitt *et al.* 2009; Aerts *et al.* 2011; Chapter 2).

Limitations of codominant microsatellite (SSRs) data analysis in allopolyploid organisms

Despite the proliferation of statistical tools over the last several years for the estimation of genetic diversity, parentage and mating system in diploid organisms, this remains a serious bottleneck for polyploid organisms. As a result, the transformation of polyploidy

molecular traits (such as codominant markers) to dominant molecular traits has been commonly practiced (for example, Sampson and Byrne 2012; Vallejo-Marin and Lye 2013; Chapter 2). Following this approach, we treated codominant markers as dominant molecular traits to estimate paternity, mating system and spatial autocorrelation of *C. arabica*. This resulted in straightforward polyploidy analyses at the cost of a potential loss of information that could be obtained through exploiting the codominant nature of the data. Moreover, our wild coffee sampling involved only one Afromontane coffee forest. Despite its limitations, our analyses provide a first approximation of the mating system and pollen flow pattern of tetraploid wild *C. arabica* in its native range. However, because of the allotetraploid nature of the *C. arabica* genome, our mating system and pollen flow analyses should be viewed and interpreted with caution, until subgenome specific markers for the putative parent species of *C. arabica* (*i.e.* for *C. robusta* and for *C. eugenioides* (Lashermes *et al.* 1999; Maurin *et al.* 2007; Tesfaye *et al.* 2007) become available.

4. 6 CONCLUSIONS

We have provided the first formal mating system analysis of *C. arabica* in its natural habitat, and we report a mixed mating system for Arabica coffee. Our results show impact of forest management on mating patterns in understory *C. arabica* populations. Pollen exchange is higher in FC populations than in SFC populations. Intensive rainforest management further promotes selfing in *C. arabica*. Although we could not find statistically significant effects of forest management on the transfer of genetic diversity between generations, our results show that, in the long term, forest management may jeopardize the conservation of genetic diversity in *C. arabica*. Our findings are of significant relevance for the ongoing *in situ* conservation efforts of wild *C. arabica* in southwest Ethiopian forests. To guarantee *in situ* conservation of the wild Arabica coffee genetic resources in Ethiopia, we recommend to (1) avoid intensification of traditional forest coffee systems to maintain diversity and abundance of the pollinator

community needed for pollen dispersal; and (2) enhance ecosystem pollination services in the managed, semi-forest coffee systems.

CHAPTER 5:

NO POLLEN LIMITATION AND HIGH REPRODUCTIVE ASSURANCE IN WILD AND MANAGED *COFFEA ARABICA* POPULATIONS IN ETHIOPIAN RAINFORESTS

This chapter has been submitted as:

Berecha G, Duchateau L, Aerts R, Brys R and Honnay O (2014) No pollen limitation and high reproductive assurance in wild and managed *Coffea arabica* populations in Ethiopian rainforests. *Submitted to Biological Journal of the Linnean Society*.

5. 1 SUMMARY

Habitat alteration may negatively affect pollinator richness and abundance, and plant visitation rates, jeopardizing successful mating through pollen limitation and pollination failure. Plant populations from altered habitats may also undergo evolutionary transitions, such as a switch from outcrossing to selfing, as selfing provides reproductive assurance (RA) when pollinators are scarce. We studied the mating system of wild *Coffea arabica* populations from Ethiopian Afromontane forests and quantified and compared pollinator visitation rates, the degree of pollen limitation, RA, and autofertility (A), in natural coffee forests (FC) and intensively managed semi-forest coffee systems (SFC). For these, different pollination treatments, including caging, supplemental hand pollination and emasculation were performed in both FC and SFC systems. The experiments took place during two coffee flowering seasons (January-March) in the study area in 2011 and in 2012. Contrary to our expectation, coffee flowers received higher pollinator visits in the SFC compared to FC sites, very likely due to the introduction of bee hives near or in the SFC systems. Nevertheless, these higher visitation rates in the SFC systems did not result in higher fruit set than in the FC system, in both study years. Fruit set was significantly higher in open pollinated flowers (outcrossing+selfing) compared to bagged flowers (selfing only). Coffee forest management did neither affect outcross- nor self pollen limitation, which both appeared to be very low in *C. arabica*. Finally, we found no evidence that fruit set in self-compatible *C. arabica* is pollen limited. Overall, our study showed that pollinators improve the productivity of *C. arabica* in its native range in Ethiopia. Therefore, it is important to maintain structural and taxonomic diversity in coffee forests, to maintain nesting sites and alternative nectar sources outside the coffee flowering season for the pollinators.

Key words: Afromontane moist evergreen forest; coffee; Ethiopia; pollination experiment

5. 2 INTRODUCTION

Anthropogenic changes of habitat quality through, for example, eutrophication or forest management can have detrimental impacts on plant-pollinator interactions both in wild plant populations and in crops (Potts *et al.* 2010; Garibaldi *et al.* 2011; Vanbergen *et al.* 2013). This is because habitat alteration may negatively affect pollinator abundance and diversity, and jeopardize successful mating through pollen limitation and pollination failure (e.g. Wilcock and Neiland 2002; Aguilar *et al.* 2006; Winfree *et al.* 2011). It is therefore of high importance to quantify the effects of habitat disturbance on pollination rates, and on the degree of pollen limitation in both wild species and crops.

Next to their direct impact on plant reproductive success, anthropogenic habitat disturbance may also have evolutionary consequences for plant species (Morgan and Wilson 2005; Eckert *et al.* 2010; Jacquemyn *et al.* 2012). Especially in specialist plant-pollinator mutualisms, an altered pollination environment and loss of pollinators following habitat disturbance may impose various selective forces on the plant mating system (e.g. Harder and Aizen 2010), yet the exact evolutionary trajectories that a species' mating system will take following anthropogenic disturbances is often difficult to predict (Eckert *et al.* 2010; Jacquemyn *et al.* 2012). One evolutionary consequence of the impoverishment of pollinator communities that has been documented relatively well, is the transition from an outcrossing breeding system to a predominantly selfing one (Eckert *et al.* 2010; Brys *et al.* 2012; Dart and Eckert 2013). Transition from outcrossing to selfing in pollinator poor environments has been documented in several plant species occurring in a large variety of habitats, including *Eichhornia paniculata* (Barett *et al.* 2009), *Centaurium erythraea* (Brys and Jacquemyn 2012), *Blackstonia perfoliata* (Brys *et al.* 2013), and *Aquilegia canadensis* (Herlithy and Eckert 2007). The most widely accepted reason why the capacity for autonomous selfing evolves is that it provides reproductive assurance (RA) (Harder and Aizen 2010; Eckert *et al.* 2010), offering successful pollination and reproductive output when scarcity of pollinators or mates limits outcrossing (Eckert *et al.* 2010).

To quantify the degree of pollen limitation, RA and the possible evolutionary transition towards a higher capacity for autonomous selfing and/or self-compatibility in pollinator poor environments, pollination experiments are crucial (Eckert *et al.* 2010). The degree of pollen limitation in plant species is commonly quantified through comparing the seed production of flowers experiencing natural pollination to the seed production of flowers supplemented with abundant outcross pollen (Knight *et al.* 2005; 2006). The capacity of RA and thus contribution of autonomous selfing to total reproductive output can be quantified experimentally using floral emasculation (anther removal) (Eckert *et al.* 2010; Dart and Eckert 2013). The extent to which autonomous selfing increases fecundity through compensating for decreased pollinator deposited pollen depends on the plants' capacity to successfully produce seed following selfing, as compared to outcrossed seed production. Since fruit and seed set can be hampered by the negative consequences of early inbreeding depression, the commonly used measure of pollen limitation based on the reproductive outcome following supplemental outcross pollination, is expected to overestimate the actual benefits of autonomous self-pollen deposition (Eckert *et al.* 2010). Therefore, studies that aim to adequately quantify RA in plant populations should include both a supplemental self-pollination treatment and an outcross-pollination treatment (Eckert *et al.* 2010).

In this study, we investigated pollen limitation and RA in *Coffea arabica*, an insect pollinated perennial woody understory shrub of Ethiopian rainforests. Wind has also been reported to have a potential role in Arabica coffee pollination (Klein *et al.* 2003a, b). *Coffea arabica* is widely recognized as a predominantly selfing species (e.g. Free 1993; Antony *et al.* 2001; Davis *et al.* 2006; 2010). However, there is some controversy among studies that reported outcrossing rates in Arabica coffee from different coffee producing regions of the world. For instance, outcrossing rates of less than 10% in Colombia (Castillo-Zapata 1976), 12% in Brazil (Carvalho and Krug 1949), 7-15% in Kenya (Van der Vossen 1974), and 40 to 60% in Ethiopia (Meyer 1965) have been reported, based on pollination studies. We studied mating patterns in wild Arabica coffee populations in Ethiopia, based on the inheritance of genetic marker variation (Chapter 2), and reported a multilocus outcrossing rate of 76%, typical for mixed mating species (Goodwillie *et al.* 2005; Busch *et al.* 2010; Zhu and Lou 2010).

Coffea arabica dominates the global coffee market today, and it also plays a significant role in Ethiopia's economy contributing over 35% of the total export value (Labouisse *et al.* 2008). It is a source of income for over one million coffee growing households and over 15 million people derive their livelihood from this crop in Ethiopia (Labouisse *et al.* 2008). The Afromontane moist evergreen forests of Ethiopia are the natural habitat of Arabica coffee and although they harbor the wild gene pool of cultivated Arabica coffee worldwide, they are highly threatened by anthropogenic activities (Getahun *et al.* 2013; Hylander *et al.* 2013). These forests have become highly fragmented and greatly exposed to varying levels of human interventions aimed at maximizing coffee productivity (Senbeta and Denich 2006; Labouisse *et al.* 2008; Aerts *et al.* 2011). The extent of these interventions vary from little or no disturbance in the so-called forest coffee systems (FC) to very intensive management in so called semi-forest coffee systems (SFC). In the SFC systems, management entails selective removal of climax tree species and understory trees and shrubs for optimum light penetration and yield maximization (Schmitt *et al.* 2009; Senbeta and Denich 2006; Aerts *et al.* 2011). In addition, the practice of introducing traditional beehives by the local community is common. The honey bee, *Apis mellifera*, is native to Ethiopia (Meixner *et al*. 2011), and traditional honey production in this part of the region depends on colonization of the hives by wild swarms of honeybees (Dietemann *et al*. 2009). Bee hives are not restricted to the forest fragments of the SFC system. Hives are also installed in solitary trees and in forest margins of the FC system, but the hive density may be higher in the SFC as farmers tend to move hives into coffee forests two weeks before coffee flowering, to take advantage of the coffee flowers' nectar for honey production.

The increasing management intensity of these forests have already been shown to significantly affect different components of local biodiversity such as epiphytic orchid diversity (Hundera *et al.* 2013a), tree species diversity (Hundera *et al.* 2013b), wild Arabica coffee genetic diversity (Chapter 2) and pollinator diversity (Chapter 3). It can thus be expected that these changes in habitat quality and pollinator diversity may have potential effects on the process of mating and reproduction in coffee. The effect of habitat disturbance and management intensity on fruit set and seed production in

Arabica coffee in plantations and in Central American agroforestry systems is well documented (Klein *et al.* 2003a; Boreux *et al.* 2011; Priess *et al.* 2007). However, data on the impact of such intensive forest management interventions on mating system functioning and fruit set in the native range and natural habitat of *C. arabica* is missing so far.

Here, we comprehensively studied the mating system of wild *C. arabica* through pollination experiments, and we assessed the degree of pollen limitation and the contribution of autonomous selfing to total fruit and seed set in both natural coffee forest (FC) and intensively managed coffee forests (SFC).

Specifically, we aimed at:

1) Quantifying the difference in pollinator visitation rates among FC and SFC management systems. We expect that pollinator visitation is higher in FC system than in the more disturbed SFC system;

2) Comparing natural fruit and seed set, and the degree of outcross- and self pollen limitation in *C. arabica* in contrasting FC and SFC management systems. We expect that fruit and seed set are lower in SFC, since abundance and diversity of pollinators and their biotic interactions are more altered, and the coffee shrubs thus more pollen limited;

3) Comparing the degree of RA and autofertility in *C. arabica* among FC and SFC systems. We expect higher RA in SFC than FC as a mechanism to compensate for low quantity of pollen delivery as a result of pollinator scarcity and altered plant-pollinators interaction.

5. 3 MATERIALS AND METHODS

Study species

Arabica or “Highland” coffee (*Coffea arabica* L.) is a perennial woody shrub belonging to the large family of the *Rubiaceae*. It is the only self-fertile, allotetraploid (2n =4x=44) species of the *Coffea* genus (Davis *et al.* 2006; 2010). *Coffea arabica* has its center of origin and diversity in the highlands of southwestern Ethiopia (Anthony *et al.* 2002). It is a shrub of the understorey of the moist Afromontane evergreen forest, where it reaches a height of 3 to 12m when unattended but when cultivated its height is often manually limited to 2m to facilitate fruit harvesting. *Coffea arabica* has a dimorphic branching habit in which vertical (orthotropic) shoots form horizontal (plagiotropic) fruiting branches that bear flowers in clusters. Under normal conditions, *C. arabica* starts flowering at an age of three years. There are 2 to 12 inflorescences, each containing four flowers per leaf axil or node. Within each flower, there are five stamens with long anthers and short filaments inserted into the corolla tube and a pistil with a long, thin style having a two-branched stigma and an inferior ovary with two chambers each containing one ovule (Free 1993; Hedberg *et al.* 2003). The stigma is receptive when a flower opens at dawn and the anthers dehisce soon afterwards. The base of the style secretes nectar which attracts different pollinator taxa, which are mainly bees in Ethiopia (Fig 5.1) (Martins *et al.* 2007; Samnegard *et al.* 2014; Chapter 3).

Figure 5.1 *Coffee arabica* flowers being pollinated by honey bees in the Afromontane moist evergreen forest of SW Ethiopia.

Once successfully pollinated, coffee fruits take 7- 9 months to reach maturity. Generally, each flower develops into a berry with two seeds (‘beans’), but sometimes only one of the two ovules develops into a seed, a condition known as pea berry (Wintgens 2012). Such types of fruits are common in the study area (personal observation) and have a detrimental effect on yield quantity and quality. Coffee flowering is triggered by rain showers after a dry spell of 3–4 months, often between January and April, and coffee shrubs flower one or two times per year. The majority of the shrubs (>90 %) flowers fully or at least partially during the first flowering period.

Study landscape

The study was conducted in two contrasting landscapes in the Jimma region, in southwest Ethiopia. Coffee forest (FC) sites were located in a large continuous moist evergreen Afromontane forest with no or little human disturbance, in the Belete-Gera National Forest Priority Area (NFPA). SFC sites were located in forest fragments east of the NFPA, in a landscape mosaic of crop land, pasture, riverine wetland, small human

settlements, isolated farmsteads and forest (Aerts *et al.* 2011). The study was conducted in two consecutive years; from January 2011 to December 2011, and from February 2012 to December 2012. A total of eight study sites were selected. Four of these sites represented highly managed semi-coffee forest fragments (SFC) less than 15km from Jimma, in the Manna district (7°43'56.9''N 36°44'50.2''E), whereas the other four sites were located in natural forest (FC) in the Gera district, 70km from Jimma (7°47'39.9''N 36°19'35.7''E). Each of the eight study sites consisted of a randomly established plot of 15m by 15m.

Pollinator visitation rate

Observations of coffee flower visitors were performed between January 2012 and April 2012. Within each of the established plots, we selected six similar sized coffee shrubs that were at a full blooming stage. Pollinators were observed during sunny days between 9:00 AM and 3:00 PM, and we observed each shrub for a period of 25 minutes. A visit to a flower was defined as a pollinator approaching and effectively touching the flower. Due to large numbers of simultaneous visitors and the high number of flowers visited by some visitors, recording visitation rates at the flower, and even at the inflorescence level was practically difficult. Therefore, visitation rate was recorded at the plant level, and we quantified visitation rate to an individual plant as the number of visits per hour (V/H) (Munyuli 2011).

Pollination experiment

The pollination experiments were designed by adapting the recently recommended approaches by Eckert *et al*. (2010) (see Fig. 5.2).

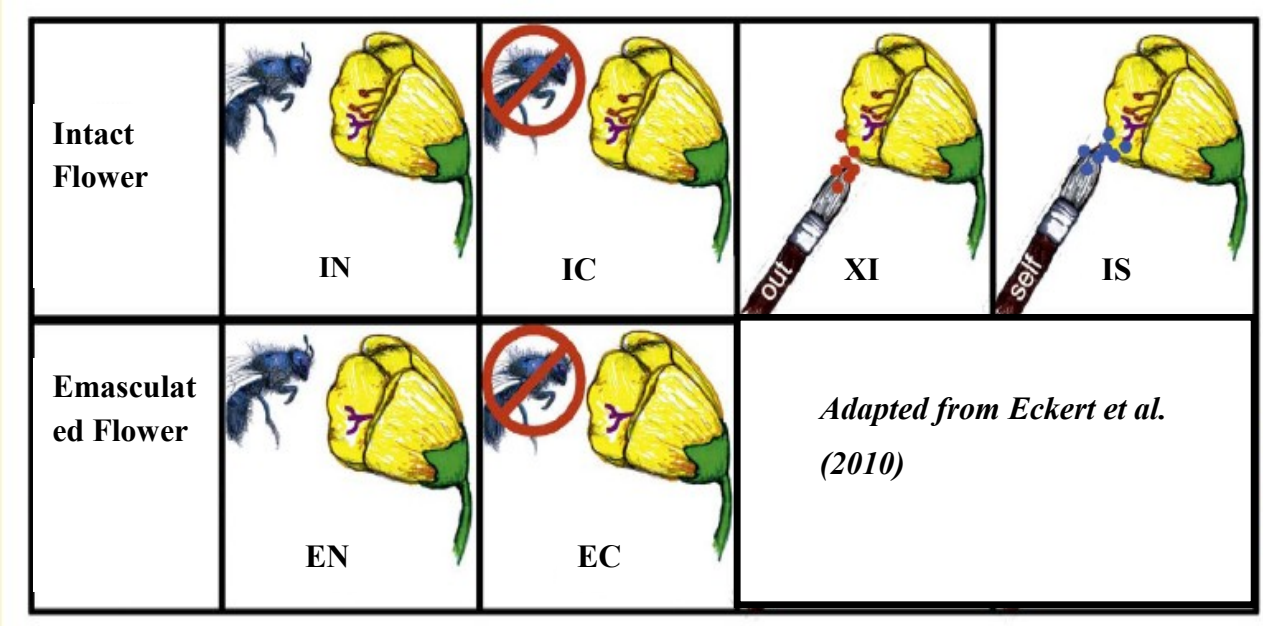


Figure 5.2 Pictorial presentation of the *Coffea arabica* flowers manipulation experiment in SW Ethiopia.

At each site, we selected the same six coffee shrubs as described above. On each plant, we carefully selected six branches of similar stage to set up six flower manipulation treatments. We paid extra attention to uniformity in the number of flowers per branch. However, the number of flowers per tree varies from one production system to the other. Shrubs in the FC system usually produce less flowers than shrubs in the SFC (the SFC management is principally carried out by farmers to boost shrub flowering rates and hence potential productivity). Therefore, on average 46 flowers were selected per branch, resulting in 1380 flowers per plot in the FC system, and on average 73 flowers per branch and 2190 flowers per plot in the SFC system. Unopened flower buds were removed. The six treatments were: (1) open intact flower (IN) (flowers were left open for both pollinators and wind); (2) bagged intact flowers (pollination is only possible via wind and autonomous selfing) (IC); (3) open emasculated flowers (EN) (emasculated flowers were open for both pollinators and wind, but selfing is not

possible); (4) bagged emasculated flowers (EC) (successful pollination is only possible via wind and is always outcrossed); (5) self pollination (IS) (supplemental hand pollination with pollen from the same plant); and (6) outcross pollination (XI) (supplemental hand pollination with pollen from other coffee shrubs). Both emasculation and bagging of mature flower buds were performed one day before anthesis. Flowers were hand pollinated by gently touching the stigma with collected anthers. For the supplemental outcrossing treatment, pollen were obtained from at least six individuals growing at a distance of 20 m from the receptive plant. All flowers were pollinated by the same person. As stigmas remain receptive for only 2-3 days, nets were removed after two weeks. To exclude the effect of crawling insects such as ants, we applied sticky glue to the main trunk of each of the selected coffee shrubs.

We quantified the effect of the different pollination treatments on (i) initial fruit set (*i.e.* the proportion of flowers that developed into a coffee berry five weeks after flowering); and on (ii) final seed set (*i.e.* the proportion of initiated fruits that produced one or two seeds within the berry, nine months after flowering).

Pollen limitation and reproductive assurance in *C. arabica*

We calculated outcross and self pollen limitation (PL_X and PL_S), the proportional increase in seed production via supplemental outcross pollination and self pollination, respectively, as [1- (IN / XI)] and [1- (IN / IS)]. Reproductive assurance (RA), the proportion of seed production attributable to autogamous self-pollination, was calculated as [1- (EN/ IN)]. The negative values were truncated to zero so that the index ranged from 0 (flowers fully pollinated) to 1 (no pollination). We also calculated autofertility (A) as the ratio between IC and IS (Eckert *et al.* 2010).

Statistical analysis

The effect of management system on insect visitation rate was quantified with a mixed model with management system as the fixed factor and plot as the random factor. Other mixed models were used to assess the effects of management system, pollination treatment and their interaction on initial fruit set and final seed; plot and coffee shrub were entered as random effects. Random error terms met the normality assumption. We

analyzed the data of the two years (2011 and 2012) separately. For the index data (PLx, PLs, RA and A), mixed model were used with plot as the random effect, and management type as the fixed effect. We performed pairwise comparisons between treatment effects using Tukeys LSD test. All the analysis were performed using SAS v. 9.3 (SAS institute 2008).

5. 4 RESULTS

Effect of management intensity on pollinator visitation rates

A total of 20 hrs of observations were made at all the study sites in the 2012 season. Significantly more pollinators visited coffee plants in SFC (on average 487 ± 6.0 SE visits per hour per shrub) compared to FC (on average 318 ± 8.5 SE visits per hour per shrub) ($F_{1,28}$ = 260.5; P < 0.0001; Fig. 5.3).

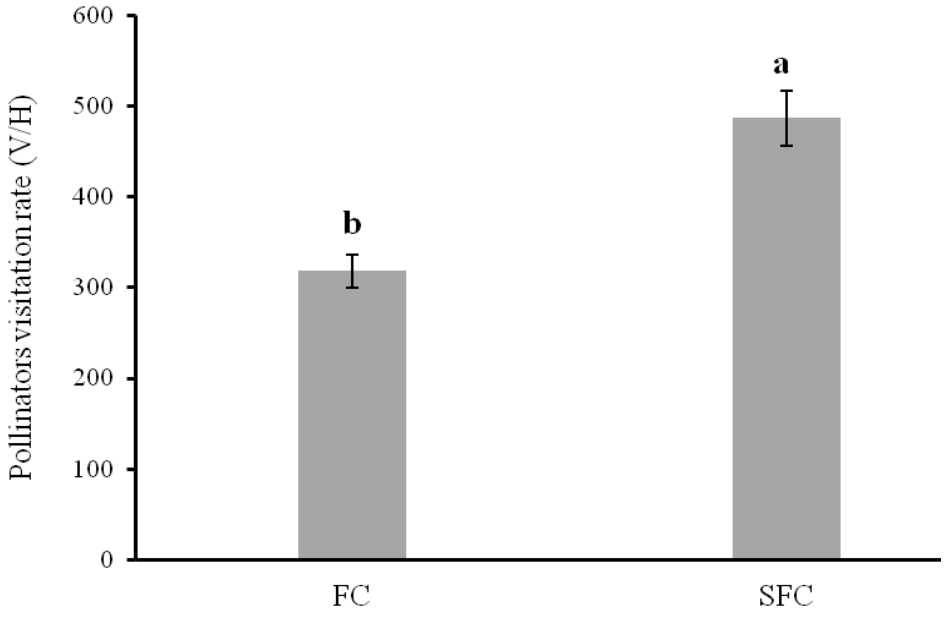


Figure 5.3 Differences in pollinator visitation rate between forest coffee (FC) and semi-forest coffee (SFC) in SW Ethiopia. Management intensity significantly affected visitation rate ($F_{1, 28}$= 260.51, P< 0.0001).

Effect of management intensity on initial fruit set

Fruit set was significantly affected by the pollination treatments in both years. The main effect of management (FC vs. SFC) and the interaction between management and pollination treatment were not significant (in both 2011 and 2012) (Table 5.1). Pairwise comparisons did not reveal significant differences between IN, XI and IS in 2011 (Table 5.2). IN resulted in significantly higher fruit set than IC, EC and EN (Fig 5.4 and Table 5.2). IC gave significantly lower fruit set compared to IN. In the 2012 season, the pairwise comparisons among treatments revealed that IS was significantly higher than IN, but the difference between XI and IS was not significant (Fig 5.4 and Table 5.2). Pairwise comparisons of IN vs. IC, and of EC vs. EN showed significant differences in terms of fruit set (Table 5. 2 and Fig 5.4).

Table 5.1 Results of mixed models of fruit and seed set for *Coffea arabica* in response to management and flower manipulation treatment in SW Ethiopia.

			Year 1		**Year 2**	
Factors, by variables	Effect*	DF	F value	*P* value	F value	*P* value
Fruit set (%)						
Management	fixed	1	0.08	0.79	0.76	0.42
Treatment	fixed	5	90.43	<0.001	157.72	<0.001
Management × Treatment	fixed	5	0.80	0.55	1.95	0.088
Seed set (%)						
Management	fixed	1	0.51	0.50	51.24	<0.001
Treatment	fixed	5	0.12	0.99	0.63	0.67
Management × Treatment	fixed	5	0.88	0.50	0.19	<0.001

* Plots and coffee shrubs were included as random effects in the mixed model.

Table 5.2 Mean fruit and seed set (± *SE*) of *Coffea arabica* occurring in both highly managed and least/unmanaged coffee forests following different pollination treatments over two years cropping season.

Pollination treatments	***2011***		***2012***	
	Fruit set (%)	**Seed set (%)**	**Fruit set (%)**	**Seed set (%)**
Open intact flowers (**IN)**	80.2 ±2.53a	89.8 ±2.22a	73.2 ±3.43b	88.1 ±0.79a
Bagged intact flowers (**IC)**	68.6 ±2.53b	90.7 ±2.22a	64.9 ±3.43c	87.4 ±0.79a
Open emasculated flowers (**EN)**	53.3 ±2.53c	90.3 ±2.22a	40.3 ±3.43d	88.6 ±0.79a
Bagged emasculated flowers **(EC)**	34.2 ±2.53d	89.8 ±2.22a	22.1 ±3.43e	88.2 ±0.79a
Self pollination **(IS)**	81.5 ±2.53a	91.4 ±2.22a	82.0 ±3.43a	88.4 ±0.79a
Outcross pollination (**XI**)	83.0 ±2.53a	90.7 ±2.22a	80.4 ±3.43ab	87.7 ±0.79a

Means followed by the same letters within one column are not significantly different at α = 0.05.

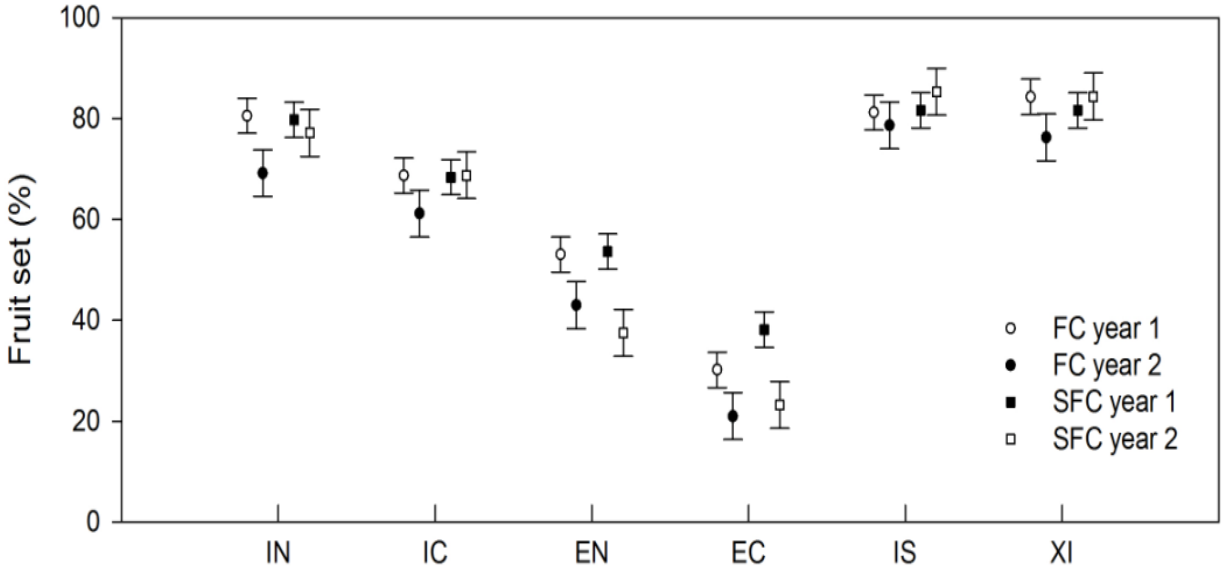


Figure 5.4 Effect of flower manipulation treatments on mean fruit set (± *SE*) in *Coffea arabica* in SW Ethiopia, in 2011 and 2012. **IN**, open intact flowers; **IC**, bagged intact flowers; **EN**, open emasculated flowers; **EC**, bagged emasculated flowers; **IS**, self-pollination, **XI**, outcross-pollination; **FC**: forest coffee system, with little or no human interferences; **SFC**: semi-forest coffee system where human interferences are very high.

Effect of management intensity on final seed set

Neither the flower manipulation treatment, the management system nor their interaction had significant effects on seed set in the 2011 cropping season (Fig 5.5 and Table 5.1, 2). In the 2012 cropping season, seed set was significantly affected by the main effect of management and by the interaction between treatment and management system, whereas no significant effect of pollination treatment on seed set could be observed (Table 5.1 and Fig 5.5). Overall, the final seed set was significantly lower in the SFC (83.8%) compared to the FC (92.3%) system. Splitting up our data set in SFC plots and FC plots yielded no significant treatment effects in the subsets.

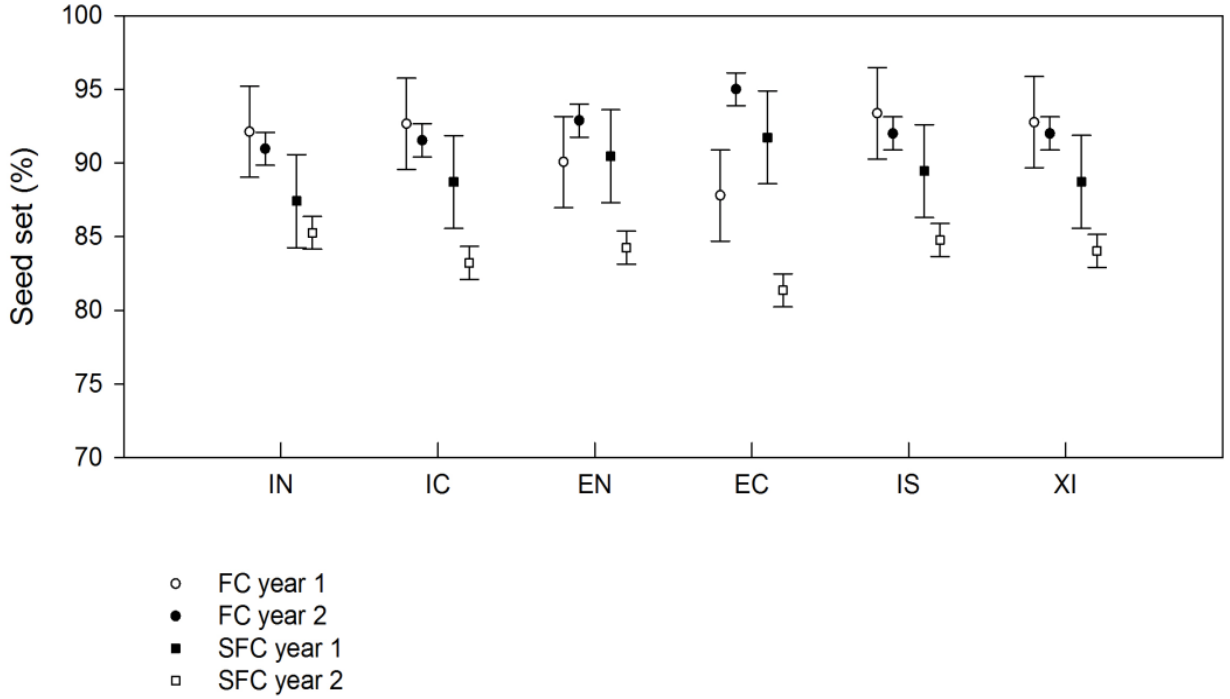


Figure 5.5 Effect of forest manangement intensity on the proportion of seed set in *Coffea arabica* in its native range. **IN**, open intact flowers; **IC**, bagged intact flowers; **EN**, open emasculated flowers; **EC**, bagged emasculated flowers; **IS**, self-pollination, **XI**, outcross-pollination. **FC**: forest coffee system, with little or no human interferences; **SFC**: semi-forest coffee system where human interferences are very high. The main effect of management was significant in 2012 ($F_{1, 28} = 51.24$, $P = 0.0004$) but not in 2011 (F= 4.84, P=0.070).

Pollen limitation and reproductive assurance

Management had no significant effect on PLx, PLs, RA and autofertility indices in coffee (Table 5.3), and overall outcross- and self pollen limitation were very low, both in 2011 and 2012 (Fig 5.6). The overall mean $\pm$ SE outcross pollen limitation was 0.096 $\pm$ 0.031 and 0.081 $\pm$ 0.027 in FC and SFC, respectively. Mean $\pm$ SE self pollen limitation was 0.074 $\pm$ 0.029 and 0.073 $\pm$ 0.026 in FC and SFC, respectively. We found mean $\pm$ SE

indices of RA of 0.369 ± 0.052 in FC and 0.426± 0.072 in SFC. Similarly, we detected a mean± SE autofertility of 0.817 ± 0.052 and 0.825 ± 0.052 in FC and SFC, respectively.

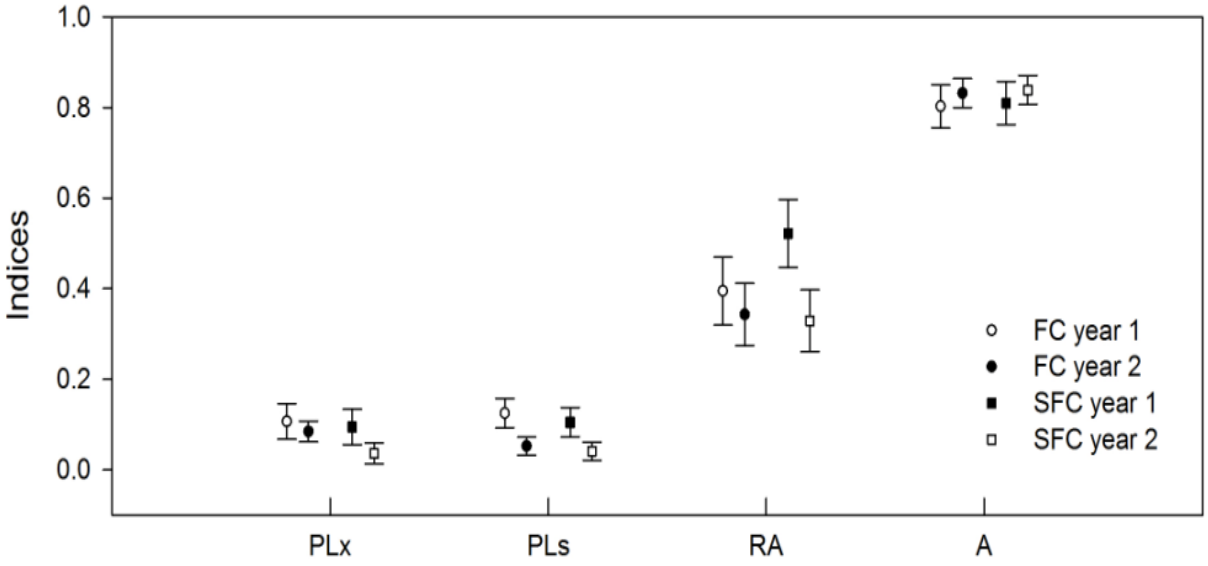


Figure 5. 6 Effect of management intensity on the index of outcross and of self pollen limitation (**PLx** and **PLs** respectively), reproductive assurance (**RA**) and autofertility (**A**) calculated from percent fruit set in the 2011 and 2012 cropping seasons. **FC**: forest coffee system, with little or no human interferences; **SFC**: semi-forest coffee system where human interferences are very high.

Table 5.3 Mixed model results for the indices of outcross pollen limitation (**PL_x),** self pollen limitation (**PL_s)**, reproductive assurance (**RA**) and autofertility (**A**), based on fruit set for *Coffea arabica* in response to management intensity in SW Ethiopia.

		Year 1		**Year 2**	
Variables	DF	F value	*P* value	F value	*P* value
PL_x based on fruit set (%)	1	2.21	0.19	0.05	0.83
PL_s based on fruit set (%)	1	0.18	0.69	0.25	0.63
RA at fruit set (%)	1	0.02	0.90	1.42	0.28
Auto fertility based on fruit set	1	0.29	0.61	0.11	0.75

* Plot was used as random effect

5. 5 DISCUSSION

Effect of management intensity on pollinator visitation

Our findings show that forest management intensity has an important effect on pollinator visitation rates in Arabica coffee. Increased habitat disturbance has often been reported to reduce richness and diversity of pollinators, and to result in altered plant-pollinator interactions (Gonzålez-Varo *et al.* 2009; Vergara and Badano 2009; Eckert *et al.* 2010). Such anthropogenically altered mutualistic interactions between agricultural crops and their pollinators have been shown to negatively affect fruit set and yield, with important economic consequences (Klein *et al.* 2007; Gallai *et al.* 2009; Garibaldi *et al.* 2011). Our results showed that coffee shrubs that were growing in the intensively managed SFC system had higher visitation rates than coffee shrubs in the relatively unmanaged and structurally and floristically complex FC system. This was contrary to our expectation,

because these more natural FC systems can be expected to support a more diverse insect pollinator community, with higher niche complementarity between species (Hoehn *et al.* 2008), as also suggested by other study results from our study area, where we found a higher expected richness of 20.7 pollinator taxa in FC compared to 12.9 in SFC (Chapter 3). Flower visitation rates and the presence of certain pollinator guilds can also be influenced by local conditions such as light intensity, distance to forest patches, time of the day, the abundance of coffee floral resources, other species flowering, and specific forest management practices employed by farmers (Munyuli 2011; 2014). Similarly human land use may also alter the relative amount of resources for pollinators in time and space (Persson and Smith 2013). In the study landscape, forest management practices for coffee cultivation in SFC systems involve the removal of shrubs other than coffee, excessive canopy thinning and gap filling through planting of wild seedlings (Aerts *et al.* 2011). This can be expected to facilitate light penetration into the shrub layer; allowing higher coffee shrubs densities, and allowing shrubs to develop more branches and abundant flower resources. This contrasts to coffee shrubs in FC systems, where coffee shrubs are sparsely distributed, are having rather the habit of sapling than of a shrub, and are bearing fewer flowers (Schmitt *et al.* 2009; Aerts *et al.* 2011). Therefore, one possible reason for the higher visitation rate observed in highly impacted SFC forest fragments is the presence of abundant flowers, attracting social insects such as honeybees. Former observations in our study area indeed showed a shift to a honey bee dominated community (Chapter 3) in SFC systems. Mass–flowering wild and/or domesticated crop species are indeed often pollinated by social bees that are able to use the abundant resources by recruiting from their colonies (Jha and Vandermeer 2009). A recent study that explored *C. arabica* pollinator in their native range already reported semi-wild honey bees as the dominant pollinators (Samnegard *et al*. 2014). Furthermore these authors reported that the abundance of honey bees was positively influenced by the amount of coffee flowers, but not by complex shade tree structures. Moreover, as honey bees are so mobile and can travel up to five kilometers from their hives (Beekman and Ratnieks 2000), they can move from one coffee forest fragment to the other, depending on the availability of flowering resources, hence increasing visitation rates.

An additional plausible reason for the honey bee dominance and the higher visitation rates in SFC systems is the common practice of introducing bee hives in and around SFC systems by local farmers. Honey production is widespread in the study area, and traditional beehives made up of split logs, carved and tied together, and scented with smoke generated from burning specific tree species are used. In the study area, farmers set out the traditional beehives by tying them to branches one to two weeks before coffee flowering to maximize the colonization of the beehives by wild honey bees' swarms. Also studies conducted in Latin America and Asia, where *C. arabica* has been introduced, showed *Apis mellifera* to be the dominant pollinators, although other eusocial bees were also frequent (Badano and Vergar 2011; Boreux *et al.* 2013). Higher bee diversity was often associated with low management intensity (Vergar and Badano 2009), proximity to natural forests (social bees) and local conditions such as higher light intensity (solitary bees) (Klein *et al.* 2003a, b; Ricketts 2004).

4.2 Effect of forest management intensity on fruit and seed set

Flower manipulation treatments had a significant effect on fruit set in *C. arabica*, irrespective of the forest management intensity. Because *C. arabica* has been reported to be a self fertile species (Davis *et al.* 2006; 2010), fruit set and productivity have traditionally been considered to be relatively independent from pollinating insects. However, our findings clearly show the importance of cross pollination as the fruit set in Arabica coffee was higher following pollen supplementation and natural open pollination, as compared to fruit set of bagged flowers where pollinators were excluded. Fruit set was consistently and significantly higher in IN compared to IC, indicating the role of pollinators in enhancing fruit and seed set. Several recent studies have also demonstrated the role of insect pollination for increasing fruit set in *C. arabica* (e.g. Klein *et al.* 2003b; Roubik 2002; Veddeler *et al.* 2008; Vergara and Badano 2009; Badano and Vergara 2011) and in other crops such as *Jatropha curcas* (Negussie *et al.* 2014). All this shows the importance of maintaining a good pollination environment as one of the good agricultural practices for maintaining productivity of coffee shrubs.

These practices should include 1) provision of nesting sites by encouraging farmers to preserve large old trees on farm or near coffee farms, and 2) pollinator friendly farming.

Forest management intensity had a positive effect on seed set in wild Arabica coffee. Seed set was significantly higher in the FC system than in the SFC system, in 2012. Seed set followed a similar trend, without a significant difference between FC and SFC, however, in 2011. The lower seed set in SFC, despite higher visitation rates, could be explained by environmental stressors that possibly limit fruit maturation, low pollinator efficiency in delivering high quality and quantity pollen, and the breeding system of *C. arabica*. As Arabica coffee is a self-fertile species, pollen supply may not be the only limitation for fruit production. True limitation of fruit and seed production by pollen supply is most likely in self-incompatible, animal pollinated plant species (Bos *et al.* 2007). This knowledge suggests that other factors might mediate seed set in *C. arabica*. Among others, anthropogenic forest management intensity can alter the microclimate within fragmented forests, degrading patch quality (Didharn and Lawton 1999; Broadbent *et al*. 2008). In such degraded forest patches, fruit maturation could be negatively affected, and the proportion of fruit set reaching maturity may be reduced. Stressors to fruit maturation such as drought, nutrient deficiencies, herbivory and within-fruits competition (e.g Knight 2005; 2006) are expected to be higher in the SFC than in the FC system, as the latter is more resilient to human perturbation than the former. Lower seed set in SFC could also be attributed to increased facilitated selfing by pollinators. In case of facilitated selfing, the proportion of fruit set reaching maturity may be reduced due to early acting inbreeding depression as self-fertilized embryos often do not survive to seed stage (Husband and Schemske 1996).

4.3 Effect of forest management on pollen limitation and reproductive assurance

Many studies have documented pollen limitation in a large number of plant species, and pollen limitation has been found to vary at different spatial and temporal scales, even within a species (Ashman *et al.* 2004). Such variation in out/self pollen limitation can be caused either by recent ecological perturbations (such as habitat fragmentation) or by the stochastic pollination environment (Wilcock and Neiland 2002; Ashman *et al.* 2004; Aguilar *et al.* 2006; Winfree *et al.* 2009). In this study we detected very low outcross and

self pollen limitation in self compatible Arabica coffee. Furthermore, pollen limitation does not appear to be affected by the intensification of the coffee management in moist evergreen Afromontane forests. The absence of increased outcross and self pollen limitation in SFC could be associated with high abundance of honeybees. Furthermore, the floral display in SFC may be higher, tempting pollinators to stay longer on the same plant, increasing self pollination through geitonogamy (Brunet and Sweet 2006; Williams 2007). Finally, the presence of low out/self pollen limitation in *C. arabica* can be explained by its capacity of both geitonogamously and autonomously self fertilization. The higher level of autofertility detected in FC and SFC (81.7% and 82.5%, respectively) in this study showed the higher contribution of autonomous selfing for total seed set. Hence, self compatibility and presence of very low pollen limitation in *C. arabica* suggests that the ability to self may provide additional RA. Indeed we detected modest RA, up to 39.5 % in FC and 52.2% in SFC.

5. 6 CONCLUSIONS

Our study showed significant impact of forest coffee management intensification on pollinator visitation rates. Contrary to our hypothesis, pollinator visitation rates were higher in highly managed coffee forest fragments (SFC system). The lower visitation rate in the least managed coffee forest (FC system) was not associated with either outcross pollen limitation or self pollen limitation. Forest management intensity influenced seed set in *C. arabica*, and higher seed set was found in FC compared to SFC (but because coffee shrubs in SFC are more numerous and bear more flowers, coffee yield is higher in SFC). Forest fragmentation and coffee management intensity had no significant effect on outcross pollen limitation, self pollen limitation, reproductive assurance and autofertility. In conclusion, *C. arabica* is not pollen limited as such in its region of origin and genetic diversity, but insect pollination clearly contributes to increased coffee production.

CHAPTER 6:

COFFEE CUP QUALITY DETERIORATES WITH INCREASING MANAGEMENT INTENSITY IN ETHIOPIAN AFROMONTANE FORESTS

This chapter is unpublished:

Berecha G, Aerts R, Vandepitte K, Roldán-Ruiz I and Honnay O (2014) Coffee cup quality deteriorates with increasing management intensity in Ethiopian Afromontane forests.

6. 1 SUMMARY

Coffee beverage quality is a function of management practices employed by farmers, soil characteristics, local microclimatic conditions, coffee genotype, processing methods, and postharvest handling operations. Anthropogenic activities in Afromontane coffee forests of SW Ethiopia have been shown to impact forest microclimate and the genetic structure of the residing *C. arabica* populations, but impacts of human activities on organoleptic quality and its attributes are not yet documented. Here, we (1) compared organoleptic quality of coffee beans from highly managed forests (SFC) with those from unmanaged forests (FC) using standard organoleptic quality assessment protocol by a panel of certified Q-grade cuppers; and (2) determined the effect of management intensity, soil variables and coffee genotype on coffee organoleptic quality. Highly managed forest fragments consistently showed lower quality scores on nearly all organoleptic attributes as compared to unmanaged forests. Neither the soil variables nor the coffee genotpes significantly affected the coffee organoleptic quality. Thus, for sustainable supply of quality coffee that command better prices and satisfy consumers' interests, intensification of natural coffee forest management through canopy thinning and introduction of coffee cultivars should be avoided to safeguard the genetic integrity and beverage quality of wild Arabica coffee in its native range in SW Ethiopia.

Key words: Coffee quality, *C. arabica,* aroma, Ethiopia, Afromontane forests, Beverage quality

6. 2 INTRODUCTION

Coffee is the leading global beverage after water, and its trade on the world market exceeded US$ 22.7 billion during 2010-11 (ICO 2012). Over 60 tropical and subtropical countries produce and export coffee, making it the main agricultural export commodity for some of them (Vieira 2008). Ethiopia is the fifth exporter of coffee in the world, after Brazil, Vietnam, Colombia, and Indonesia in that order (ICO 2013). Globally, over 100 million people derive their livelihood from coffee (Waller *et al.* 2007). Out of the 124 described coffee species (Davis *et al.* 2006; 2010; 2011), the commercial production depends only on two species, *Coffea arabica* and *C. canephora* (Anthony *et al.* 2002; Labouisse *et al.* 2008). Arabica or highland coffee (*C. arabica* L.; Rubiaceae) accounts for 66% of the global coffee production, whereas the remaining proportion comes from *C. canephora* (Labouisse *et al.* 2008). Arabica coffee is known for its beverage quality, aromatic characteristics, and low-caffeine content and therefore commands higher prices on the international market, as compared to *C. canephora* which is characterized by a stronger bitterness, and higher-caffeine content (Gielissen and Graafland 2009; ICO 2013).

C. arabica is the only coffee species grown in Ethiopia and the country is the primary centre of its origin and genetic diversity (Anthony *et al.* 2002; Vega 2008). In Ethiopia, coffee plays a central role in the social, economic and cultural life, as it contributes to over 35% of the total export value (ICO 2012), and as over 25% of the citizens are directly or indirectly dependent on coffee, i.e. being involved in its production, processing or marketing (Labouisse *et al.* 2008).

The Ethiopian moist evergreen montane forests host wild Arabica coffee populations (Senbeta and Denich 2006; Schmitt *et al.* 2009; Aerts *et al.* 2011), but these forests are threatened by excessive deforestation, fragmentation and intensive forest management for coffee cultivation (Gole *et al.* 2008; Labouisse *et al.* 2008; Hundera *et al*. 2013a). Four traditional coffee production systems with clear variation in management intensity are used in Ethiopia (Teketay 1999). These are forest coffee (FC), semi-forest coffee (SFC), garden coffee and plantation coffee (see Labouisse *et*

al. (2008) for more detail). The intensity of coffee management gradually increases from FC over SFC and garden coffee, to plantation coffee (Schmitt *et al.* 2009; Aerts *et al.* 2011). FC systems are characterized by an almost undisturbed forest structure with a dense canopy cover, and a deeply shaded forest understory (Senbeta 2006). In this system, due to low light intensity, the vegetation cover in the forest understory is low and the herb layer is not well developed. Density of coffee shrubs is very low because coffee is not as competitive as many other shrub and small tree species under low light conditions. As a result, the majority of coffee shrubs is thin and tall, has a very low growth rate and carries few fruits (Schmitt *et al.* 2009). In the SFC system, the forest structure is highly disturbed because farmers generally remove a significant proportion of the canopy trees and most understory vegetation. Due to such removal of shading canopy cover and competing vegetation and because farmers transplant seedlings from elsewhere, the coffee shrub density increases, the coffee shrubs grow larger, and coffee yields are much higher than in the FC systems. In some households, farmers introduce improved coffee cultivars which are provided by governmental institutions (Chapter 2). Unlike FC and SFC systems, plantation coffee production system use recommended fertilizers, chemicals for the control of pests, and improved cultivars.

Because forest management by farmers has a strong influence on the forest microclimate and on the amount of berries the coffee shrubs are able to produce, it is expected that it has consequences for coffee berry quality too. The final beverage quality of the coffee bean is known to be influenced by many factors, including soil factors, local microclimatic conditions, coffee genotype, processing methods, and postharvest handling operations (Bertrand *et al.* 2006; Leroy et al. 2006). Soil quality (physical, chemical and biological) of the Ethiopian coffee forests can also be expected to be directly influenced by forest management (Slagle *et al.* 2004), as changes in light availability and temperature on the forest floor may have profound influences on decomposition rates and nutrient cycling (Chen *et al.* 2000). Senbeta (2006) and Kufa (2006) showed that the soil is acidic to slightly acidic with limited phosphorus content in the natural habitats of wild Arabica coffee. Van Der Vossen (2009) indicated that better quality, acidic Arabica coffee is often produced on soils of volcanic origin, with pH raging

between 5 and 6. Several studies showed that soil nutrient availability is one of the important factors controlling biochemical composition and beverage quality of coffee beans (Mazzafera 1999). Abebe *et al.* (2008) studied the influence of soil properties on cup quality of wild Arabica coffee beans from forests of Ethiopia and showed better cup quality with increased level of available phosphorus and potassium. They also found improved aroma with higher levels of pH, Mg, Mn, and Zn. The altered microclimate, including higher insulation and lower air humidity (Vaast *et al.* 2006), within small fragmented coffee forest fragments may also directly affect coffee beverage quality (Beer *et al.* 1998; Muschler 2001; Vaast *et al.* 2005). However, empirical evidence that demonstrates the effect of shade on beverage quality through its influence on micro-climatic conditions (temperature, humidity etc.) is sparse (but see Vaast *et al.* 2006; Van Der Vossen 2009; Bosselmann *et al.* 2009).

In addition to forest management which alters soil characteristics, human activity has also changed the genetic structure of the coffee individuals through introduction of cultivars into coffee forests (Chapter 2). Because berry components that are involved in taste development such as alkaloids are known to deter herbivores (Zheng and Ashihara 2004), it is possible that there is selection on genes involved in pathways influencing berry chemical composition and taste, such as the biosynthesis of caffeine, chlorogenic acid and amino acids. This may imply that the degree of hybridization between the wild coffee individuals and the introduced cultivars affects coffee beverage quality from Ethiopian montane forests. Higher variation in terms of caffeine content among *C. arabica* accessions was reported by Dessalegn *et al.* (2008), along with its negative association with acidity, body, flavor and overall standard.

The general objective of this study was to assess the effect of anthropogenic disturbance on organoleptic quality of wild coffee from SW Ethiopian montane forests. Our first specific aim was to compare the organoleptic quality of wild coffee originating from coffee forests differing in management intensity. Our second aim was to specifically evaluate the effects of management, soil characteristics and coffee genotypes on organoleptic cup quality of wild coffee.

6. 3 MATERIALS AND METHODS

Description of the study area

The study was conducted in the Jimma region in southwestern Ethiopia, from September 2012 to July 2013. The study involved 20 study sites. Seven sites were randomly located in one of the last remaining large natural continuous forests, in the Gera district 70km west of Jimma (7°47'39.9''N 36°19'35.7''E). Each study site here consisted of a 5ha forest block, representing the coffee forest production system (FC). Six more of such forest blocks were selected from large highly managed forest fragments (SFC) in the Manna district, 15km west of Jimma (7°43'56.9''N 36°44'50.2''E). The remaining seven study sites were located in seven small forest fragments (4-9ha) in the Manna district. All together, thirteen sites from Manna district were sampled representing highly managed semi-forest coffee production system (SFC).

Study species

Arabica coffee is the only allotetraploid (2n = 4x = 44), formed by relatively recent natural hybridization between the putative parents, *C. canophora* and *C. eugenioides* (Lashermes *et al.* 1999). Wild Arabica coffee of Ethiopia is geographically isolated from all other species in the genus (Silvestrini *et al.* 2007). Wild coffee generally occurs between 1500 and 1900m a.s.l, but cultivated plants are found over a much wider range, between 1000 and 2800m (Gole *et al.* 2008; Labouisse *et al.* 2008). In *C. arabica*, flowering is triggered by rain, with a short annual period of synchronous flowering usually between January and April. The species is self-compatible. *C. arabica* fruits take 7-9 months to reach maturity depending on locality and altitude, with usually longer periods (close to one year) in higher altitude areas. The population density of *C. arabica* varies with forest management intensity, with on average 3,900 individuals (≥ 0.5m in height and dbh ≥ 2cm) ha^{-1} in FC (Schmitt *et al.* 2009), as compared to 5,000 individuals (≥ 0.5m in height and dbh ≥ 2cm) ha^{-1} in the SFC in the study area (Aerts *et al.* 2011).

Sample collection and DNA extraction

In each forest fragment/block we randomly established a sampling plot of 20m x 20m. Within each plot, we sampled coffee leaves and berries from 25 randomly selected coffee shrubs. Leaves were dried on silica gel. Prior to DNA-extraction, leaves were freeze-dried for 48h and homogenized with a mill (Mixel Mill MM 200, Retsch®, Haan, Germany). Genomic DNA was extracted from 20mg homogenized leaf material using the NucleoSpin® Plant II kit (Machery-Nagel, Düren, Germany), with slight modifications of the standard CTAB protocol. We increased the incubation time during cell lysis to 60min at 65°C and we also used a two-step elution procedure incubated at 70°C for optimal recovery of bound nucleic acids.

SSR genotyping

Twenty-four microsatellites (SSRs) described in Chapter-2 were amplified in six multiplex PCRs, using a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems®, CA, USA) and a total sample volume of 10μL containing 5μL Qiagen® Multiplex PCR Master Mix (Qiagen, Valencia, CA), 2μL sample/template DNA and 0.2μL of each primer (reverse and forward, 10μM) in the multiplex combination complemented with RNase-free Milli-Q water. The multiplexes had equal thermocycling profiles with an initial *Taq* DNA polymerase heat-activation step at 95°C for 15min; 25 cycles of 30s at 94°C (denaturation step), 90s at 57°C (annealing step) and 60 s at 72°C (extension step); and a final extension step of 30min at 60°C. Then, 1μL of the PCR reaction was added to a solution of 8.8μL formamide and 0.2μL of the Applied Biosystems GeneScan^{TM} 500 LIZ® size standard. Sized fragments were scored using GeneMapper® v 4.0 (Applied Biosystems).

Analysis of genetic data

Due to limitations of the software that can handle allotetraploid data, we converted the co-dominantly scored data to a dominant dataset comparable to the output of an Amplified Fragment Length Polymorphism (AFLP) marker approach, and based on the scoring of each individual allele as present or absent. We used the R package

POLYSAT (Clark and Jasieniuk, 2011) as a central data handling facility, particularly for importing the SSR data from the GeneMapper® software and for converting the data.

We estimated among population genetic differentiation (Φ_{PT}) based on Euclidian genetic distances (Huff *et al*. 1993). The molecular variance (MV) and Φ_{PT} resulted from a hierarchical analysis of molecular variance (AMOVA) on the dominantly scored data set as performed in GENALEx 6.41(Peakall and Smouse 2006; 2012). For AMOVA, management system (FC vs. SFC) was used as a regional grouping factor. To assess the association between coffee genotypes and organoleptic quality attributes, we performed a Principal Coordinate Analysis (PCoA) based on the pairwise Φ_{PT} matrix in GENALEx 6.41(Peakall and Smouse 2006; 2012).

Soil sampling

Soil sampling was performed at 0-30 cm depth. Seven soil samples per plot were collected using an auger, and were bulked and thoroughly mixed to yield one composite soil sample per plot. A total of 20 composite soil samples (one composite soil per plot) were analyzed for the different soil variables at the national soil analysis laboratory in Addis Ababa, Ethiopia. Soil variables analyzed and the used methodology are summarized in Table 6.1.

Table 6.1 Soil characteristics and soil analysis methods used in the study

Soil characteristic	Method
Texture	Hydrometer
pH(H_2O)	Potentiometric-water extract
Electrical conductivity	Conductivity in water extract
Available Boron (Av. B)	Azomethine -H- Colorimetric
Available Phosphorous (Av. P)	Olsen *et al.* and Bray II
Available potassium (Av. K)	Ammonium acetate extract-Flame photometry
Cation exchange capacity (CEC)	Sodium equivalent by flame photometer
Exchangeable Calcium (Ca) and Magnesium (Mg)	Ammonium acetate extract -Flame AAS
Exchangeable sodium (Na) and Potassium (K)	Ammonium acetate extract-Flame photometry
Organic carbon (OC)	Walkley-Black
Total Nitrogen (TN)	Kjeldahl
Micro nutrients (Mn, Fe. Cu, Zn)	DTPA Extract- Flame AAS

Coffee berry sampling and post-harvest processing

In order to not to introduce bias, harvesting and post-harvest procedures were kept constant by uniform procedures. All coffee samples were harvested during the peak harvest period of September-October 2012. From each study plot, 6kg red ripe coffee berries were harvested. To maintain uniformity, all harvested samples were subjected to sorting by the same person and any under and over ripe berries were discarded. The wet method of coffee processing was employed (Wintgens 2012), and the cherries were manually pulped using a small scale drum pulper with a capacity of 45-50kg cherries per h. Due to the difficulty in adjusting drum pulpers based on berry size, approximately 3-5% of the beans were discarded. The pulped samples were allowed to ferment in separate 15l buckets for 24h before they were washed manually. After thorough washing, the beans were soaked for 6h in clean water. Afterwards, the samples were separately dried on raised mesh wire under natural sun and samples were covered with plastic sheet during midday, night time and early in the morning to avoid parchment

cracking and moisture regain, respectively. Approximately, 1kg dried parchment coffee sample from each plot was obtained and stored in separate perforated plastic bags and transported to Belgium for sensory evaluation.

Assessment of coffee organoleptic quality

Coffee quality assessment was entirely based on visual and sensory evaluation by expert coffee cuppers. The final coffee quality score was the sum of two types of assessments: green coffee (raw bean) analysis (account for 40 % of the final score point) and cup taste (liquoring) which account for 60% of the final score point. Green coffee analysis involves visual inspection of physical characteristics of the coffee bean, and includes a screen analysis which makes a size assessment, defect count, appearance or color test and shape evaluation, which usually refers to the structure of the beans. For both physical and sensory analysis, 350g of coffee beans per plot was taken. The sample was equally divided into three: 1/3 for raw, 1/3 for cup taste and 1/3 as a reference sample. The coffee bean samples from all plots were assessed for both physical and organoleptic quality attributes following the procedures of the Specialty Coffee Association of America (SCAA). The coffee samples were assessed by a panel of Q-grade SCAA-certified professionals of Efico (www.efico.com) in Belgium. For cup taste, 120g green coffee beans were roasted and ground per sample per plot. Cup quality tests were performed on an infusion prepared with 12g ground coffee in 250ml water. To avoid bias, samples were presented randomly and identities of the samples were not known to the panel of cuppers. Three certified Q-grade cuppers tasted five cups of infusion for each sample. Then the consensus quality score of the three cuppers was recorded for each sample. The evaluated organoleptic attributes included aroma, body, acidity, flavor, aftertaste, uniformity, balance, sweetness, cup cleanness and total quality score. All quality attributes were rated from 1 (very poor) to 10 (outstanding) except total score which was rated from 0 to 100 where coffee beans with total score of 80 and above according to SCAA are considered as specialty coffee that command better price (www.scaa.org).

Statistical analysis

To reduce the multidimensionality of the soil dataset, a Principal Component Analysis (PCA) with varimax rotation was performed on the soil variables. Similarly, a varimax rotated Principal Coordinates Analysis (PCoA) was performed on the genetic data based on the pairwise population Φ_{PT} genetic distance matrix. We then used a multiple regression model to relate the organoleptic attributes (dependent variables) to management (FC vs. SFC), the first principal component of the soil variables, and the first principal coordinate of coffee genotypes. Finally, the different organoleptic quality attributes were compared between the FC and SFC using independent samples t-test. All the analyses were performed using SPSS V 20.

6. 4 RESULTS

Influence of forest management on coffee genotypes and soil properties

The first principal component ($PC1_{soil}$) axis generated from the soil variables explained 33.2% of the total variation. Based on the factor loading score, soil chemical properties such as pH, available potassium, calcium, magnesium, total potassium, manganese and zinc contributed greatly to $PC1_{soil}$ (Appendix Table 6.1). On the other hand, of all evaluated soil variables, only pH, available K, CEC, total K, Na and Cu were significantly influenced by management system (Appendix Table 6.2).

The first principal coordinates ($PCoA_1$) axis generated from the genetic calculated on the pairwise population Φ_{PT} genetic distance matrix, explained 35.6% of the variance. Overall among-population genetic differentiation was high (Φ_{PT} = 0.213, P<0.001) with a genetic differentiation of 0.045 (Φ_{PT}) between management systems (FC vs. SFC).

Influence of management, genotype and soil properties on organoleptic quality of *C. arabica*

Our multiple regression analysis showed that management (FC vs. SFC), soil variables and genetic data significantly predicted all assessed organoleptic quality attributes, except aroma (Table 6.2). Nevertheless, only management (FC vs SFC), not genotype or soil, added statistically significant to the prediction of the assessed organoleptic quality attributes in the regression model. Flavor, aftertaste, body and total quality scores were significantly and negatively influenced by coffee management intensity ($P < 0.05$; Table 6.2)

Table 6.2 Linear regression model outputs of the association among coffee management (FC vs. SFC), first principal component axis generated from soil variables ($PC1_{soil}$), first principal coordinate axis generated from coffee genetic data on 500 individuals from 20 stands (*$PCoA_1$),* and different organoleptic quality attributes of *Coffea arabica* in its native range, SW Ethiopia.

Regressors	***Aroma***		***Flavor***		***Aftertaste***		***Acidity***		***body***		***Total score***		
	t	***Pr***	***t***	***Pr***	***t***	***Pr***	***t***	***Pr***	***t***	***Pr***	***t***	***Pr***	***VIF***
Management	-0.392	0.700	- 3.68	0.002	- 3.64	0.002	- 2.98	0.009	-5.35	<0.001	- 3.58	0.002	4.23
$PCoA_1$	-0.96	0.354	2.02	0.060	1.947	0.069	1.11	0.285	0.76	0.458	0.99	0.334	2.04
$PC1_{soil}$	1.21	0.224	1.11	0.283	2.05	0.057	-0.251	0.805	1.02	0.321	1.74	0.101	1.82
R^2	**0.242**		**0.582**		**0.646**		**0.405**		**0.765**		**0.755**		-
Overall _F value	**1.71**	**0.206**	**7.42**	**0.002**	**9.75**	**0.001**	**3.63**	**0.036**	**17.35**	**<0.001**	**10.97**	**<0.001**	-

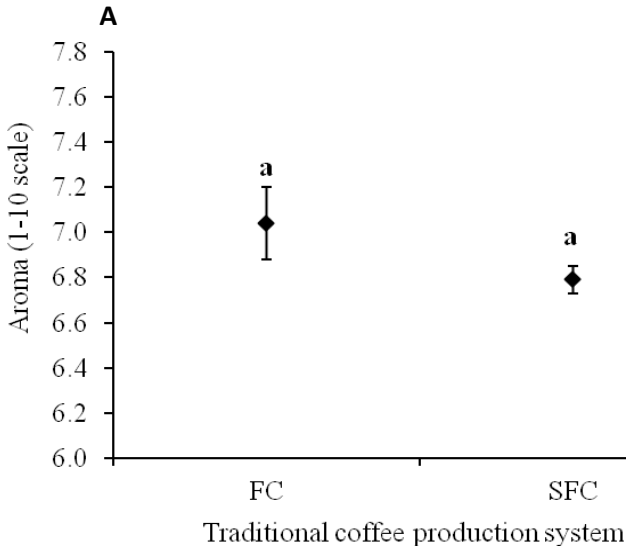
VIF= variance inflation factors; t = student t-test value; *Pr* = probability value

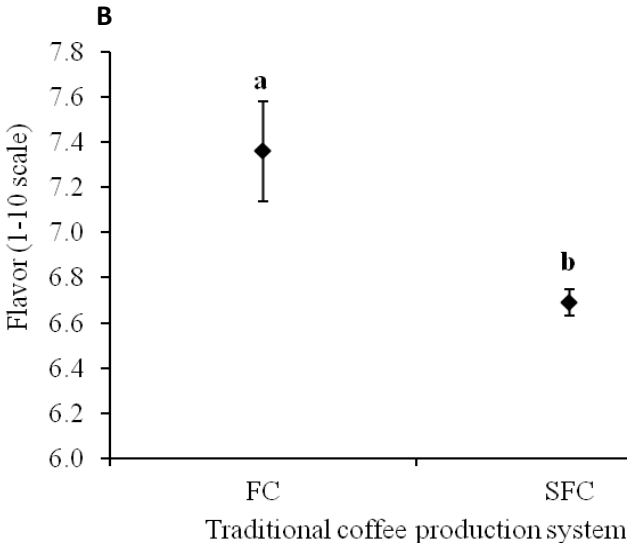
Effect of forest management intensity on organoleptic quality attributes

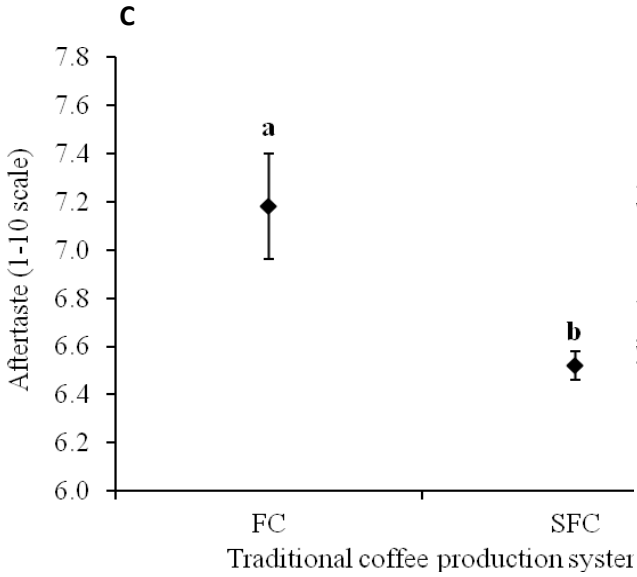
We found a significant effect of forest management intensity on most organoleptic quality attributes of wild Arabica coffee (Table 6.3). Both coffee beans originating from FC and SFC received the same scores for uniformity, balance, cup cleanness and sweetness. However, except for aroma, the other evaluated organoleptic quality attributes (flavor, aftertaste, acidity, body and total quality) received significantly higher scores in FC than SFC (Fig. 6.1).

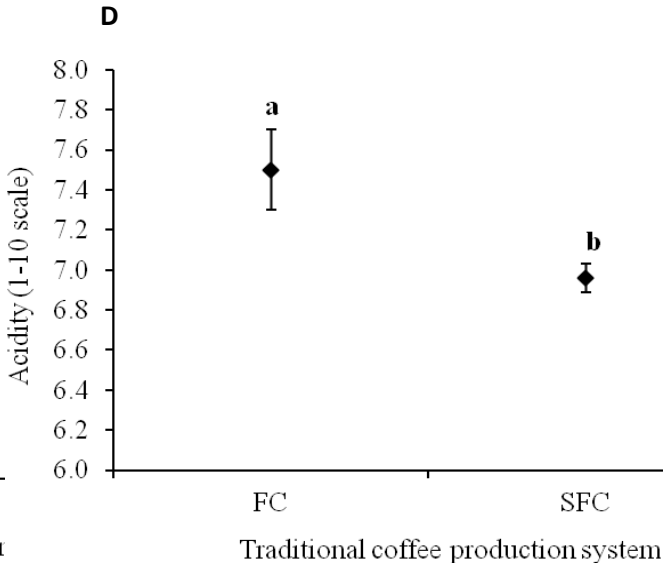
Table 6.3 Means and standard error (S.E.) of the organoleptic quality attributes of wild Arabica coffee in its native range, SW Ethiopia. The data were organized into two management systems: unmanaged forest coffee (FC) and highly managed semi-forest coffee (SFC).

Organoleptic quality attributes	FC (n =7)		SFC (n = 13)			
	mean	*S.E*	*mean*	*S.E*	*t-value*	*P-value*
Aroma	7.04	0.16	6.78	0.06	1.74	0.099
Flavor	7.36	0.22	6.69	0.06	3.63	0.002
After taste	7.18	0.22	6.52	0.06	3.76	0.001
Acidity	7.50	0.20	6.96	0.07	3.18	0.005
Body	7.36	0.35	6.52	0.05	7.10	<0.0001
Uniformity	10.00	0.00	10.00	0.00	0.00	1.000
Balance	8.00	0.00	8.00	0.00	0.00	1.000
Cup cleanness	10.00	0.00	10.00	0.00	0.00	1.000
Sweetness	10.00	0.00	10.00	0.00	0.00	1.000
Total score	81.71	0.76	78.40	0.35	4.58	<0.0001


A
Aroma (1-10 scale)
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
a
a
FC
SFC
Traditional coffee production system


B
Flavor (1-10 scale)
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
a
b
FC
SFC
Traditional coffee production system


C
Aftertaste (1-10 scale)
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
a
b
FC
SFC
Traditional coffee production syster


D
Acidity (1-10 scale)
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
a
b
FC
SFC
Traditional coffee production system

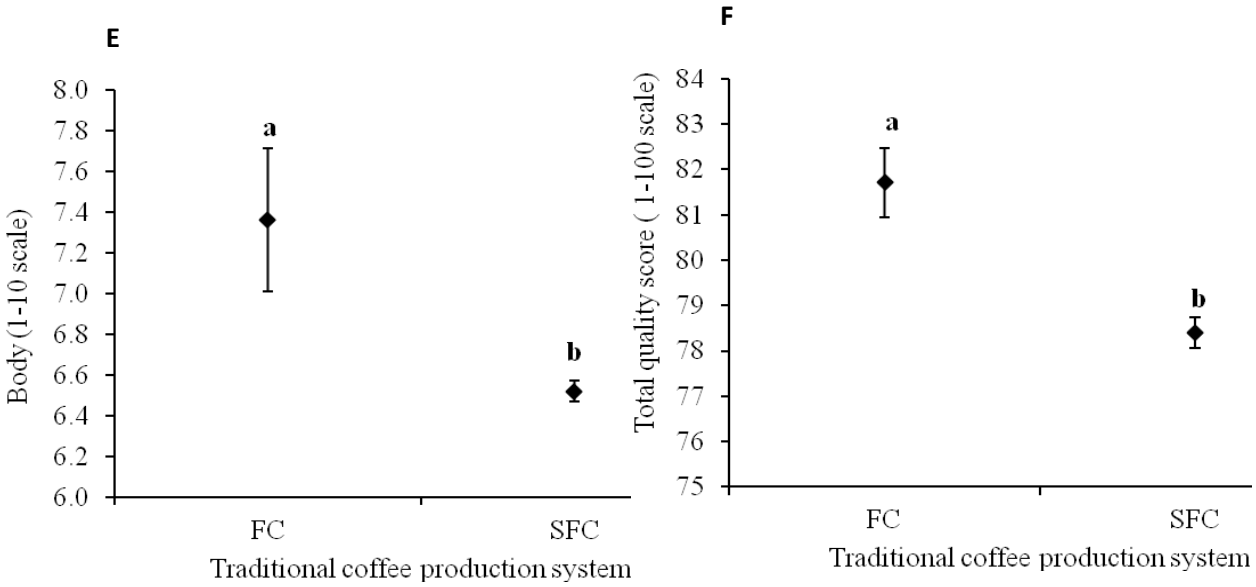


Figure 6.1 Effect of coffee forest management intensity on aroma (A), flavor (B), aftertaste (C), Acidity (D), Body (E) and total quality score (F) of wild Arabica coffee in SW Ethiopia. ***FC***: forest coffee production system; ***SFC***: intensively managed semi-forest coffee production system (SFC). Bars capped with same letters are not significantly different from each other at α=0.05.

6. 5 DISCUSSION

Effect of forest management on beverage quality

In agreement with our hypothesis, results showed the influence of anthropogenic forest disturbances on organoleptic quality of wild *C. arabica* in its native range. Anthropogenic forest disturbances, in addition to their impacts on species richness and species diversity (Morriss *et al.* 2010), can alter habitat quality (Wright 2005). In SW Ethiopia, human pressure on the forest resources in the form of forest disturbance and forest management for coffee cultivation is very high. Recent studies conducted in the same study region already demonstrated the impact of forest management for coffee

cultivation on tree species diversity and stand structure (Aerts *et al.* 2011; Hundera *et al.* 2013b), epiphitic orchid species diversity (Hundera *et al.* 2013b), wild coffee genetic diversity and integrity (Chapter 2), and insect pollinator diversity (Chapter 3). For all these components of forest biodiversity, diversity significantly declined with increasing forest management intensity, i.e. high diversity in FC and low diversity in SFC systems. These studies attributed the observed patterns to altered habitat quality inside the forest fragments, altered biotic interactions (e.g. plant-pollinator interactions), removal of conspecific flowering species, reduction of nesting sites for pollinators, and anthropogenic introduction of improved cultivars. The lower quality scores for all organoleptic quality attributes evaluated in SFC compared to FC in the current study could be attributed to altered micro-climatic conditions (increased sunlight and wind, increased temperature, and decreased in humidity) inside highly managed coffee forests, thereby aletering the biochmical composition of the beans (Didharn and Lawton 1999; Broadbent *et al*. 2008). Didharn and Lawton (1999) also found lower canopy height, a higher rate of evaporative drying, lower leaf litter moisture content and lower litter depth in fragmented forest patch compared to continuous forest. Farmers excessively thin shade tree canopy and understory shrubs to enhance coffee productivity in SFC systems (Schmitt *et al.* 2009; Aerts *et al.* 2011).

Management also resulted in variation in the level of shading between FC and SFC system. Such management practices aimed at boosting productivity may not equally support better product quality, however. Indeed this is what we observed. Highly managed forest fragments (SFC) showed lower quality scores than least managed forests (FC). Coffee maturation can be affected by environmental factors such as light because during the course of seed maturation, various quality influencing components are synthesized or chemically altered, producing a unique flavor, acidity and taste (Senyuva and Gokmen 2005). Most studies that compared coffee quality between shade and sun grown coffee concluded that shade grown beans are better in quality than sun grown ones (Vaast *et al.* 2006; Geromel *et al.* 2008; Van Der Vossen 2009). Furthermore, coffee shrubs grown in less shady conditions also produce more flowers and fruits with reduced bean size of lower quality, owing to competition among fruits for

available carbohydrates (Cannell 1975; Bosselmann *et al.* 2009). At moderately high altitudes (1700m a.s.l.), shading usually delays maturity of coffee fruits, and improves bean quality (Muschler 2001; Vaast *et al.* 2005; Van Der Vossen 2009). It has been shown that incomplete maturation often results in bitterness and astringency in cup quality which is often the case in coffee grown under unshaded conditions (Vaast *et al.* 2005).

Effects of genotypes and soil properties on organoleptic quality attributes of *C. arabica*

The contribution of genotypes to beverage quality of wild Arabica coffee was not significant in the studied system. This was not expected. The highly managed SFC systems that have experienced anthropogenic human activities for many years would have been expected to show higher genetic differentiation from FC populations. This was not the case, the genetic differentiation between SFC and FC detected in this study was very low (Φ_{PT} = 0.045). Indeed our genetic analysis of SFC and FC systems in Chapter 2 also revealed no significant difference between SFC and FC populations attributable to anthropogenic introduction of improved CBD resistant cultivars by framers in the highly managed SFC systems. The SFC genotypes had more affinity to the CBD resistant gene pool (Chapter 2). The low cup quality scores in SFC systems could be attributed to anthropogenic introduction of cultivars by farmers. Although the CBD resistant cultivars were selected from coffee accessions collected from different Afromontane forests containing wild Arabica coffee gene pool as an immediate solution for the catastrophic outbreak of CBD in 1970s, they were not evaluated for their quality profile. However, an early report by Bellachew *et al.* (2000) indicated that the Arabica coffee flavor profile is locality specific, and coffee beans from different locations have different flavors. For example, mokka flavor for the Harar locality, spicy flavor for Sidama, fruity flavor for Nekamte/Wellega and winy for Limu (Labouisse *et al.* 2008). Therefore, introduction of CBD resistant cultivars originally collected from other localities, may have contributed to low quality performance of coffee from SFC systems.

Our analysis did not show a statistically significant contribution of soil chemical properties to organoleptic quality attributes. This was unexpected as macro and micro-nutrients that contributed significantly to $PC1_{soil}$, such as pH, available potassium, Mg and total potassium content, were expected to influence the total quality score. This contradicts the findings of Abebe *et al.* (2008) who reported a significant association between coffee quality and available potassium and pH in coffee forests of Ethiopia. Although there was a significant difference between FC and SFC in terms of pH, available K, CEC, total K, Na and Cu, inclusion of these nutrients in our regression model did not significantly predict the organoleptic quality attributes. On the other hand, our result could lead us to believe that forest disturbances have not yet resulted in a significant difference in soil nutrient contents between managed and unmanaged forests to the level that organoleptic quality attributes are affected. This could be the case because, in SFC systems, farmers leave the weeds and shrubs they cut within the fragments to improve the fertility of the soil, and use them as mulching materials to conserve soil moisture during the dry season.

6. 6 CONCLUSIONS

This study demonstrates that forest management intensity associated with coffee cultivation influences organoleptic quality of wild Arabica coffee. Organoleptic quality attributes decreased with increased intensity of forest coffee management. Best quality beans that qualified for specialty coffee grade were obtained from FC, as compared to SFC systems. Attributes of organoleptic quality were more variable in FC than SFC systems, indicating ample opportunity for future national and international improvement programs. Neither the genotypes nor the soil chemical variables significantly contributed to enhanced organoleptic quality in the studied system (FCvs.SFC). Therefore, for sustainable supply of high quality coffee beans that fetch better prices and satisfy consumers' interest, intensification of forest coffee should be avoided. Besides, farmers should avoid introduction of a cultivar gene pool in SFC system to safeguard the genetic integrity and beverage quality of wild Arabica coffee in its native range, SW Ethiopia.

CHAPTER 7:

GENERAL CONCLUSIONS AND RESEARCH PERSPECTIVES

7.1 GENERAL CONCLUSIONS

This PhD dealt with the genetic diversity, pollination ecology and organoleptic characteristics of *Coffea arabica* L. in Ethiopian moist forests of different management intensity. In this concluding chapter the major findings of the PhD research are briefly presented and discussed, and practical recommendations regarding the conservation of wild Arabica coffee are drawn. The chapter concludes with a range of suggestions for further research towards the *in situ* conservation of wild Arabica coffee.

7.1.1 Main results

Afromontane forests of Ethiopia constitute the native habitat for many species, including wild *C. arabica* (Senbeta and Denich 2006; Gole *et al.* 2008; Schmitt *et al.* 2013). Wild populations of Arabica coffee in these rainforests are genetically diverse, and likely possess desirable traits that can be used to improve the cultivated varieties of *C. arabica* worldwide. The importance of these wild Arabica populations are expected to increase in the future, as plant breeders attempt to address the threats of the combination of global environmental changes and a higher demand for food (Foley *et al.* 2011). However, these wild coffee populations are potentially threatened by excessive deforestation and the resulting forest fragmentation, forest habitat disturbance and hybridization with introduced coffee cultivars (Teketay 2001; Gole 2003; Gole *et al.* 2008). Therefore, this work aimed to broaden our understanding of the effects of each of these potential threats on wild coffee (*C. arabica* L.) genetic diversity and integrity (Chapter 2 and 4), pollination and reproductive success (Chapter 3 and 5), and cup quality (Chapter 6).

Coffee cultivation and utilization has been deeply rooted in the tradition and culture of Ethiopian society for centuries (Gole *et al.* 2008). In Ethiopia, the traditional forest bound coffee cultivation has long been considered as a biodiversity-friendly organic production system, and hence, as sustainable in the long term. This notion stemmed from the fact that coffee shrubs are growing under the canopy of naturally occurring shade tree species and that the intensity of human interventions is very restricted in FC systems, and rather modest in SFC systems, as compared to

intensively managed coffee plantations with sparse shade trees. However, in this manuscript, we showed that the capacity of Ethiopian Afromontane moist evergreen forests to support the genetic diversity, genetic integrity, and taste of the residing coffee shrubs may be compromised in the long term.

Table 7.1 Summary of major results of the study

Criterion	***FC vs. SFC***	***Indicator***
Genetic diversity (Ch.2)	=	$H_{E,C}$, *MV*
Genetic differentiation(Ch.2; Ch. 6)	yes	Φ_{RT}, *PCoA*
Similarity to cultivar genotype (Ch. 2)	<	*h-index, structure*
Pollinator beta diversity (Ch.3)	<	Number of genera
Pollinator expected diversity (Ch. 3)	>	*MaoTao*
Differentiation of pollinator communities (Ch. 3)	yes	NMS
Selfing rate (Ch. 4)	<	Number of selfed offspring
Pollen flow (Ch. 4)	>	Paternity assignment
Potential for outcross pollination (Ch. 4)	=	Multilocus outcrossing rate t_m
Fine scale spatial genetic structure (Ch.5)	No	Autocorrelation coefficient *r*
Intergenerational diversity transfer	<	$H_{E,C}$, *H'c*
Fruit set (Ch.5)	=	Proportion of flowers developed to fruits
Seed set (Ch.5)	>	Proportion of fruits containing seed
Outcross and self pollen limitation (Ch.5)	=	Proportional increase in seed production
Reproductive assurance (Ch.5)	=	Proportion of seed resulted from autogamous self pollination
Pollinator visitation rate(Ch.5)	<	Number of flower visitors
Bean cup quality (Ch.6)	>	Consensus score of cuppers

FC = unmanaged forest coffee system; SFC= highly managed semi-forest coffee system

7.1.1.1 Effect of forest fragmentation and forest management on wild Arabica coffee genetic diversity and integrity

Anthropogenic forest fragmentation and forest management are expected to reduce genetic variability of species through reducing population size, increasing genetic drift, reducing gene flow and increasing inbreeding (Aguilar *et al*. 2008). We tested this prediction for wild *C. arabica* shrubs residing in fragmented coffee forests (SFC) and those in unmanaged forest coffee (FC) in SW Ethiopia. The expected genetic diversity difference between populations from fragmented SFC systems and populations from unmanaged coffee forests (FC) was not present (Chapter 2). However, we detected strong genetic differentiation between intensively managed (SFC: Φ_{PT} = 0.176, SE 0.018) and unmanaged coffee populations (FC: Φ_{PT} = 0.131, SE 0.014). Moreover, the genetic integrity of SFC population was found to be affected in SFC systems (Chapter 2), as these populations were generally more related to the pool of introduced CBD-resistant genotypes than the FC populations. These patterns were explained by the anthropogenic introduction of both wild genotypes and CBD-resistant cultivars in SFC systems. These introductions may have offset losses of genetic variation attributable to genetic drift and inbreeding in small intensively managed coffee forests, but on the other hand, mixing cultivars with original coffee genotypes resulted in a significant signal of admixture, with a higher mean admixture coefficient in SFC (h_{SFC} = 0.74) than in FC (h_{FC} = 0.30) populations. It is important to note, however, that the apparent presence of alleles from CBD resistant cultivars gene pool in some wild populations, could also be explained by the fact that CBD resistant cultivars were derived from forest coffee genotypes collected from different parts of the country, and some of the alleles shared between FC and cultivar gene pool are expected to be identical by descent. Generally, however, we can conclude that only coffee populations from the few remaining large and more or less natural forests with a FC cultivation system are safe from the introduction of CBD-resistant cultivars and subsequent hybridisation and loss of genetic integrity. This indeed points to the need for national and international efforts to safeguard the wild coffee gene pool from loss of genetic integrity due to introduction and planting of improved cultivars (see section 7.2).

7.1.1.2 Effect of forest management on mating pattern and pollen flow in Wild Arabica coffee

Forest management and forest fragmentation had no significant influence on the outcrossing potential of Arabica coffee populations (Chapter 4). Nevertheless, we reported multilocus outcrossing rate as high as 76% in wild Arabica coffee in its native region which contrast with the established knowledge that *C. arabica* is a predominantly selfing species. Our result described in Chapter 4 showed the significant influence of fragmentation and management intensity on pollen exchange, and more pollen exchange was found in FC populations than in SFC populations. In addition, forest management reduced long distance pollen dispersal, but it enhanced selfing rates in coffee shrubs residing in fragmented forests (SFC). This pattern may be explained by the difference in floral resources between coffee shrubs growing under the two management systems. Shrubs in the FC system usually produce fewer flowers than shrubs in the SFC system. The presence of a large number of flowers in SFC is expected to tempt pollinators to stay longer on the same flower, increasing the chance of self pollen deposition, hence increasing selfing (Brunet and Sweet 2006, Williams 2007). Moreover, the presence of large number of flowers is expected to attract social insects such as honey bees that recruit their colony when resources are abundant (Jha and Vandermeer 2009). Indeed, we found higher visitation rate, with a pollinator community that was entirely dominated by honey bees in the SFC systems (Chapter 3). On the other hand, fine scale spatial genetic structure was not present neither in SFC nor in FC populations (Chapter 4), suggesting high seed dispersal in FC populations, and intense berry harvesting and coffee planting in the managed populations (SFC) (Chapter 2).

7.1.1.3 Impact of forest management on pollinator diversity and reproductive output in Wild Arabica coffee

Management practices employed by farmers are expected to influence the diversity of coffee pollinating insects by altering the plant-pollinator interaction (Klein *et al*. 2008). On the other hand, a recent review by Ngo *et al.* (2011) claimed a strong study bias towards Latin America and Asia, with few work from Africa. Furthermore, there is no one

single systematic scientific study on the pollinator community of *C. arabica* in Ethiopia. Therefore, we have surveyed potential pollinators of wild Arabica coffee, and we have also provided evidence that anthropogenic activities may negatively affect the diversity of this coffee pollinator community (Chapter 3), potentially compromising the important ecosystem service that pollinators render, and general ecosystem health (Vanbergen *et al.* 2013). More specific, we studied whether increasing forest management intensity and fragmentation resulted in potential impacts upon coffee pollination services through examining shifts in insect communities that visit coffee flowers in FC systems on the one side, and in both small and large forests with an SFC cultivation system on the other side. *C. arabica* flowers were visited by a wide range of potential pollinators, covering 10 insect orders. The most abundant taxonomic groups that were present on coffee flowers were honey bees, butterflies and hoverflies. The taxonomic richness of the coffee flower visiting insects significantly decreased, and the pollinator community composition significantly changed, with increasing forest management intensity and forest fragmentation. These results may be explained by altered micro-climatic conditions and the absence of suitable microsites for nesting within the intensively managed small forest fragments. In addition to forest thinning, tree cuttings are very frequent in SFC systems, particularly in densely populated areas, limiting the abundance of alternative conspicuous flowering plants that may provide nectar outside the coffee flowering season. Taxonomic and structural homogenization was also reported for other taxonomic groups such as trees, epiphytic orchids and arbuscular mycorrhiza in the same intensively managed coffee forest fragments (Hundera *et al.* 2013a, b; De Beenhouwer *et al.* 2014). With increasing management intensity, the taxonomic composition of the flower visiting insects community shifted towards a honey bee dominated one, likely due to the introduction of traditional bee hives in the most intensively managed forest fragments. The impoverishment of the insect communities through increased forest management and fragmentation may potentially decrease the resilience of the coffee production system in the long term, as pollination increasingly relies on honey bees alone (Ricketts *et al.* 2008; Garibaldi *et al.* 2013), which is not desirable under the current climate change scenarios (Rader *et al.* 2013).

In Chapter 5 we compared pollinator visitation rates, pollen limitation, reproductive assurance, and autofertility between unmanaged coffee forests (FC) and intensively managed semi-forest coffee systems (SFC), through *in situ* flower manipulation experiments for two consecutive cropping seasons (2011 and 2012). Intensification of coffee management impacted pollinator visitation rates. SFC populations received more pollinator visits than FC populations, which was not expected. The higher visitation rates in the SFC systems did not result in higher fruit set, however, and this was associated with higher abundance of honey bees through introduction of bee hives in and around forest fragments (Chapter 3). These findings support an important argument that the presence of higher taxonomic diversity (Chapter 3) *per se* does not guarantee increased flower visitation and subsequent fruit set rates. The environmental conditions during flowering time and the presence of other flowering plant species in the surroundings may also influence visitation rate (Potts *et al.* 2010; Winfree *et al.* 2009). Furthermore, coffee forest management did neither affected outcross nor self pollen limitation. Both outcross and self pollen limitation were very low in *C. arabica*. Similarly, forest fragmentation and coffee management intensity had no significant effect on outcross pollen limitation (PLx), self pollen limitation (PLs), reproductive assurance (RA) and autofertility (A). Fruit set was higher in open pollinated flowers than bagged flowers (pollinators excluded), indicating the importance of pollinators in enhancing fruit set and subsequent productivity of *C. arabica.*The role of insect pollinators in increasing fruit set and yield of *C. arabica* was well documented (Klein *et al*. 2003b; Roubik 2002; Veddeler *et al*. 2008; Vergara and Badano 2009; Badano and Vergara 2011).

7.1.1.4. Impact forest management on organoleptic quality of C. arabica

Forest management intensity significantly and negatively influenced organoleptic beverage quality of wild Arabica coffee (Chapter 6). Coffee from SFC systems consistently showed lower scores in nearly all evaluated organoleptic quality attributes than coffee from FC systems. Excellent beverage quality beans with a specialty coffee rank, according to SCAA standards, were obtained from FC populations only. The decline in coffee beverage quality may be attributed to major changes in forest structure

and shrub and canopy species composition (Schmitt *et al.* 2009; Aerts *et al.* 2011), and to subsequent forest microclimate changes (humidity, sunlight radiation, air circulation and temperature) in SFC systems. Altered environmental conditions within small and highly managed forest fragments may induce stress on coffee shrubs and compromise their final bean quality. Particularly, excessive thinning of the shade tree canopy exposes coffee shrubs to more sunlight, encouraging overbearing and incomplete maturation. Incomplete maturation often results in higher bitterness and astringency of the coffee beverage (Vaast *et al.* 2005). Although significant effects of soil characteristics and genetic make-up on beverage quality were expected (Abebe *et al.* 2008) this was not evident from our results. Perhaps, forest disturbances may not have resulted yet in a significant difference in soil nutrient contents between managed and unmanaged forests to the level that organoleptic quality attributes are affected.

7. 1. 2 Coffee conservation in the 'land sparing' and 'land sharing' context

The increasing demand for food and other agricultural products worldwide has resulted in both the conversion of natural habitats to anthropogenic land uses and the intensification of use of land already under agriculture (Foley *et al.* 2005). Intensification of land use for food production is often detrimental for the general environment and biodiversity (Balmford *et al.* 2012). Likewise, delineating a dedicated area from land otherwise used for food crop production or other purposes for conservation of wild species obviously raises competing claims between food production and biodiversity conservation. As a result, reconciling food production and biodiversity conservation remains a challenge for conservation biologists and agronomists worldwide.

Land sharing and land sparing are the two contrasting approaches currently advocated to reconciling food production and biodiversity conservation (Phalan *et al.* 2011; Green *et al.* 2005). While the former involves integrating biodiversity conservation and food production on the same land, using biodiversity-friendly farming methods; the later involves separation of land for conservation from land for crops, with crop land use intensification facilitating the protection of remnant natural habitats from agricultural expansion (Fig. 7.1, Phalan *et al.* 2011; Green *et al.* 2005).

The traditional forest bound SFC system in SW Ethiopia is a complex agro-forestry system with relatively high conservation value. When we contextualize land sharing strategies to the traditional SFC production systems of Ethiopia, it fails to remain an option due to the following reasons: 1) the small landholding size (0.25-4ha) of the majority of the smallholder farmers' calls for intensification 2) attempting a productivity increase through excessive thinning of shade trees and removal of understory shrubs, typical for the traditional SFC system, will definitely degrade biodiversity, and question the biodiversity claimed to be conserved (Hundera *et al.* 2013a, b; De Beenhouwer *et al.* 2013; 2014), and (3) the current government plan to double the productivity of coffee by 2015 (www.mofed.gov.et) through maximizing input utilization efficiency and scaling up of best practices.

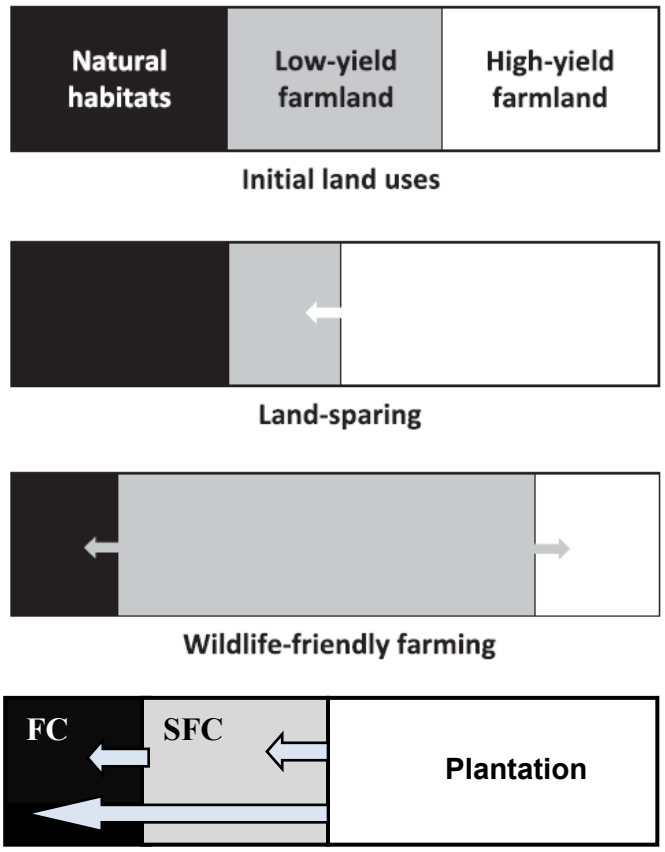


Figure 7.1 Blocks illustrating how land sparing or wildlife-friendly farming strategies could be used to meet an increase in food demand. In this illustration starting from a region with equal areas of natural habitats, low-yield farmland and high-yield farmland (top). Land sparing (second from top) involves increasing yields in the production landscape while protecting or restoring natural habitats. Wildlife-friendly farming (second from bottom) involves expanding the area of low-yield farmland at the expense of natural habitats (adapted from Phalan *et al.* 2011). * In Ethiopia FC is converted to SFC and plantation, SFC to plantation.

On the other hand, land sparing is advocated as a more promising strategy for minimizing negative impacts of food production, at both current and anticipated future levels of production (Phalan *et al.* 2011). Taking in to account (i) the current 5 year growth and transformation plan of the Ethiopian government which sets the target of doubling the productivity of coffee by the end of the period (www.mofed.gov.et); (ii) the

significance of the wild gene pool for future improvement of the worldwide cultivated varieties of *C. arabica*, and (iii) the necessity to further ensure conservation of forest inhabiting organisms (including endemic species), land sparing strategies seems more promising.

7. 1. 3 Guidelines for C. arabica conservation in Ethiopia

To ensure the *in situ* conservation of Arabica coffee genetic resources in Ethiopia we recommend to:

1) Implement multi-site *in situ* conservation approaches that can ensure sustainable conservation and utilization of the existing genetic resources.

2) Establish buffer zones of SFC surrounding more strict reserves of FC in the last remaining large forest blocks in the region/ country.

3) Avoid intensification of traditional forest coffee systems as intensification threatens the genetic integrity of the gene pool by exposing wild genotypes to cultivars. Furthermore, decreased pollen dispersal and increased selfing in *C. arabica* in SFC may increase the risk of genetic erosion.

4) Avoid establishing plantations with foreign coffee cultivars in the centre of origin of *C. arabica*.

5) Establishment of plantation coffee farms should consider some reasonable distance from the *in situ* conservation area to possibly control the gene flow from plantation coffee farms to the wild gene pool.

6) Certification of FC production could be a means to enhance the market situation and to exploit the currently available niche market with a premium price for certified organic products. Certification schemes that take into consideration the biodiversity within the protected area for *in situ* conservation should be implemented.

7) Limit the entrance of local communities to the conserved sites to reduce their impacts through coffee management or forest resource utilization. This can be done

by developing alternative livelihood improving practices that go in harmony with biodiversity conservation such as eco-tourism development; establishing management schemes involving the government and local communities including community bylaws.

8) Design fair and equitable sharing mechanisms for the benefits arising from the forest and biodiversity resources conserved therein.

9) In the already significantly managed SFC systems, sustainable intensification schemes that may at least conserve the remaining biodiversity while boosting productivity should be exploited. To do so the following practices can be suggested:

    - focusing on good agronomic practices [pruning (maintenance pruning within season, rehabilitation of old shrubs through stumping), shade regulation, mulching, weeding and cultivation] that enhance productivity with little or no negative effect on the existing biodiversity;
    - re-filling gaps (increase density of coffee shrubs) with seedlings originating from the same fragments or using Ethiopian cultivars that did not undergo a very strict process of selection, and adapted to the agro-ecology under consideration;
    - integrating apiculture with SFC production to enhance ecosystem pollination services and livelihood improvement
    - establishing flowering plants that can serve as refuge for pollinators (nesting sites) and alternative nectar sources outside coffee flowering season should be promoted.
    - encouraging farmers to establish small exclosures in the SFC systems to promote regeneration of shade tree seedlings or to spare healthy shade tree seedlings during the annual slashing activity in areas where coffee shade trees are getting old and dying.

7. 1. 4 Current conservation of wild Arabica coffee genetic resources in Ethiopia

Conservation of plant genetic resources can be accomplished using two basic conservation strategies: *ex-situ* and *in-situ* conservation (Engelmann *et al.* 2007). According to the Convention on Biological Diversity, *in situ* conservation is defined as the conservation of the ecosystems and the natural habitats and the maintenance of recovery of viable populations of the species in their natural surroundings. *In situ* conservation can be accomplished on farmers' fields, on pasture lands and in national parks or other types of natural reserves. The primary aim of on-farm *in situ* conservation is to conserve the biodiversity of traditional crop varieties in the area where they adapted or evolved. It is one of the most important *in situ* conservation methods where farmers' knowledge and traditional practices are exercised (Engelmann *et al.* 2007). *In situ* conservation offers the possibility of conserving a high diversity of species and gene pools simultaneously, as plants continue to evolve following gradual changes in their environment (Maxted *et al.* 1997). Moreover, it is a sound strategy for the conservation of non-orthodox-seed species such as *C. arabica* (Engelmann *et al.* 2007). However, there are drawbacks of this conservation strategy: (1) the conserved plant materials are vulnerable to natural and human-induced challenges, (2) they are less accessible for use, (3) the amount of genetic diversity that can be conserved is not easily measurable (4) there are high cost associated with incentives and law enforcement, and (5) it requires high levels of active supervision and monitoring.

Ex-situ conservation as an alternative strategy to *in situ* conservation, involves the conservation of components of biological diversity outside their natural habitat. The *ex-situ* conservation strategy includes techniques such as seed storage, *in vitro* storage, DNA storage, pollen storage, field gene bank and botanic garden conservation (Engelmann *et al.* 2007). In *ex-situ* conservation approach such field gene banks, populations of plants cultivated *ex-situ* are usually small, thus exposing them to many of the risks that are also faced by very small and fragmented populations, including genetic erosion because genetic variability is increasingly reduced by the combined effects of reduced gene flow and genetic drift, increased inbreeding and accumulation of deleterious mutations (Aguilar *et al.* 2008; Schoen and Brown 2001).

In Ethiopia, both discussed conservation strategies (*in situ* and *ex-situ*) have been used in a complementary way in order to conserve the coffee genetic resources. To date, ca. 11, 881 Arabica coffee accessions have been collected from different parts of the country and have been *ex-situ* conserved on research plots at the Jimma Agricultural Research Center (JARC) and its ten sub-centers, and at the field gene banks of the Institute of Biodiversity Conservation at Choche, Limu, Ethiopia (Taye 2009). Regarding *in situ* conservation efforts, the Ethiopian government, together with its international partners, has identified ca.10 Afromontane evergreen moist forests harbouring the wild Arabica gene pool for conservation. Out of these, the government has registered two sites as UNESCO Biosphere Reserves, namely the Yayu Coffee Forest Biosphere Reserve and the Kafa Forest Biosphere Reserve. However, compared to increasing anthropogenic threats, the higher diversity expected and the value of this gene pool for future crop improvement programs worldwide, much is still remaining. Some of the limitations of past conservation efforts are: i) lack of core collections for those accessions collected and *ex-situ* conserved in the field gene backs (ii) lack of systematic evaluation of the genetic diversity present using state of the art techniques (iii) the conserved materials are less known by the scientific community compared to international collections and (iv) the potential value of the gene pool is not fully estimated.

7. 2 Shortcomings and Research Perspectives

7. 2. 1 Shortcomings of the study

One obvious limitation of our work is related to the allopolyploid nature of the study species, and the application of SSRs. The allotetraploidy nature of *C. arabica* results in limited flexibility of data analysis, such as parentage analysis, mating system analysis, and quantification of spatial genetic structure based on codominant marker information. The application of codominant microsatellite markers (SSRs) in polyploidy species is limited by the difficulty of identifying true genotypes for partial heterozygotes at one locus from double homozygote at orthologous loci. Despite the proliferation of statistical tools over the last several years to handle codominant marker data in diploid organism,

this remains a serious bottleneck for allopolyploid organisms. This forced us to transform SSR data to presence/absence data. This resulted in straightforward analyses at the cost of a potential loss of information that could be obtained through exploiting the codominant nature of SSRs. Because of this, our results should be confirmed once subgenome specific markers for the putative parent species of *C. arabica* (*i.e.* for *C. robusta* and for *C. eugenioides* (Lashermes *et al.* 1999; Maurin *et al.* 2007; Tesfaye *et al.* 2007) become available. However, this type of analyses for codominant markers in polyploid species (e.g. *Dipteryx panamensis)* has been commonly practiced and is in general terms highly reliable, provided that the number of loci considered is large enough (for example, Hanson *et al.* 2008; Sampson and Byrne 2012; Vallejo-Marin and Lye 2013).

Another shortcoming that could have been avoided was the sample size (number of mother bushes sampled as source of progeny array per population). This has obviously imposed a bottleneck to the data set and limited the statistical power to detect intergenerational transfer of genetic diversity in wild Arabica. Although different studies used different sampling sizes, ranging from 5 to 15 mothers per population, depending on the level of accuracy needed and the species under study, ours was at the lower bound due to logistics requirement. On the other hand our data is still robust because we used relatively large number of loci (24 SSRs) compared to the recommended 5-10 SSRs by Ashley (2010) in her critical review. Therefore, similar future studies should consider a higher number of mother shrubs per population to minimize possible effect of researcher induced genetic bottleneck.

The third shortcoming of this study was imposed by the lack of experts and specialized laboratories in Ethiopia capable of identifying pollinators to lower taxonomic classification (species level). We were not able to send our samples to a specialized laboratory elsewhere due to lengthy process to get legal permission from the competent authority in Ethiopia.

The fourth shortcoming of this study was related to sampling bias. For some chapters (Chapter 3, 6) we sampled more plots in SFC than in FC systems. This

disproportionate sampling was adopted to account for management intensity variation among farmers, as the small sampled forest fragments are owned by different farmers. As much as possible, we accounted for the sample size variation in our analyses, but we cannot rule out the impact of this sampling bias on our results.

The fifth shortcoming was related to the lack of a taste evaluation for CBD resistant cultivars, currently introduced by the government. The main reason was that it was not possible to collect berries from the 24 CBD resistant cultivars that have been grown under similar growing conditions. Furthermore, from a logistic point of view, cup quality evaluation for an additional 24 samples on top of the 20 samples from different management categories was not possible.

7. 2. 2 Research Perspectives

This PhD work has generated important information regarding the impact of forest management intensity on genetic diversity, mating patterns, pollinator abundance and diversity, and cup quality. However, there are issues that still require the attention of future research.

1. To further increase our understanding of (i) the (functional) genetic diversity of wild Arabica coffee occurring in different Afromontane moist evergreen forests and (ii) the diversity existing within coffee Arabica accessions conserved in *ex-situ* field gene banks, more studies using state of the art molecular techniques such as Single Nucleotide Polymorphism (SNP) and Diversity Array Technology (DArT) are required. Population genomic screening using more advanced tools such as SNPs or DArT markers will allow to unravel the impact of adaptive processes on population genetics.

2. Our formal mating analysis has shown long distance gene flow (by pollen) in the unmanaged forest coffee systems. Gene flow among populations could be effected through pollen and seed. However, research work addressing seed dispersal of wild Arabica coffee in its natural habitat is still missing. We recommend future studies on seed dispersal and seed dispersal agents of coffee in Afromontane evergreen moist forests of Ethiopia.

3. We showed that pollen supplementation can increase fruit set in coffee Arabica. We also reported considerable fruit set through autogamous self fertilization in Arabica coffee. However, the extent to which autonomous selfing increases seed production depends on the survival of self versus outcrossed fertilized embryo to seed maturation, as self-fertilized embryos might not survive to the seed stage in plant having the potential for outcrossing (Husband and Schemske 1996). Therefore, it is imperative to study the germination capacity and fitness of seeds/seedlings resulting from outcross *versus* selfing.

4. Most natural habitats support many wild pollinators, providing resilient and complementary ecosystem services such as pollination (Garibaldi *et al.* 2011; Vanbergen *et al.* 2013). Our exploratory study also showed that Afromontane forests of Ethiopia harbor diverse pollinator species. Therefore, to better understand the role of pollinators in coffee pollination and pollinator response to anthropogenic human activities the following points need the attention:

    - Detailed identification of obligate vs. opportunistic Arabica coffee pollinating insect species to lower taxonomic classification (species level);

    - assess the pollination efficiency and flying behavior of key Arabica coffee pollinating insect species;

    - assess the endurance of key pollinators of wild Arabica coffee across a gradient of forest fragmentation and degradation;

    - assess landscape-scale impacts of multiple interactions (habitat fragmentation, introduction of alien species) on pollinator density and foraging behaviors

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APPENDICES

Appendix Table 1.1 Overview of the sampling locations in two contrasting landscapes in Jimma region, SW Ethiopia. Two management systems: large continuous forest coffee system (FC) and highly fragmented and managed semi-forest coffee (SFC); and three fragment categories: large natural unmanaged forest (LNF: > 100,000ha), large highly managed forest fragment (LMF: > 100ha) and small highly fragmented and managed forest fragments (SMF: 4-9ha) were sampled.

Sampled stands	**Long (E)**	**Lat (N)**	**Elev (m)**	***Sampled for***	***Management system***	**Fragment intensity**
Afalo (A1)	36.2163	7.6400	1887	Ch. 3	FC	LNF
Afalo (A2)	36.2117	7.6238	1850	Ch. 3	FC	LNF
Afalo (A4)	36.2067	7.6395	1987	Ch. 2,3,4,6	FC	LNF
Afalo (A6)	36.2092	7.6404	1889	Ch. 2,3,6	FC	LNF
Afalo (A7)	36.2072	7.6152	1718	Ch. 3	FC	LNF
Afalo (A10)	36.2241	7.6307	1825	Ch. 2,3,4,6	FC	LNF
Qacha (Q1)	36.3342	7.7829	1909	Ch. 3, 5,6	FC	LNF
Qacha (Q2)	36.3334	7.7797	1970	Ch. 3,5	FC	LNF
Qacha (Q3)	36.3313	7.7817	1920	Ch. 2,3,5,6	FC	LNF
Qacha (Q6)	36.3275	7.7837	2008	Ch. 3,5,6	FC	LNF
Qacha (Q11)	36.3238	7.7868	2108	Ch. 2,4,6	FC	LNF
Qacha (QY)	36.3432	7.7865	1926	Ch. 2	FC	LNF
Fetche (F1)	36.7482	7.7144	2085	Ch. 2,3,5,6	SFC	LMF
Fetche (F2)	36.7496	7.7150	1987	Ch. 3,6	SFC	LMF

Fetche (F3)	36.7513	7.7156	1996	Ch. 3,6	SFC	LMF
Fetche (F4)	36.7526	7.7162	1989	Ch. 3,6	SFC	LMF
Fetche (F5)	36.7545	7.7170	2026	Ch. 3,6	SFC	LMF
Fetche (F6)	36.7562	7.7168	2043	Ch. 3	SFC	LMF
Fetche (F7)	36.7634	7.7077	1882	Ch. 3	SFC	LMF
Fetche (F8)	36.7617	7.7106	1908	Ch. 2,4,5,6	SFC	LMF
Fetche (F9)	36.7617	7.7087	1882	Ch. 3	SFC	LMF
Fetche (F10)	36.7600	7.7099	1908	Ch. 3	SFC	LMF
Garuke (G1)	36.7496	7.7348	2022	Ch. 3	SFC	SMF
Garuke (G2)	36.7472	7.7318	2015	Ch. 3	SFC	SMF
Garuke (G3)	36.7458	7.7312	2017	Ch. 3	SFC	SMF
Garuke (G5)	36.7400	7.7345	2052	Ch. 3,6	SFC	SMF
Garuke (G6)	36.7422	7.7354	2031	Ch. 3	SFC	SMF
Garuke (G8)	36.7391	7.7346	2025	Ch. 3,6	SFC	SMF
Garuke (G9)	36.7387	7.7371	2042	Ch. 3,6	SFC	SMF
Garuke (G10)	36.7420	7.7368	2025	Ch. 2,3,4,5,6	SFC	SMF
Garuke (G11)	36.7477	7.7373	2040	Ch. 2,3,5,6	SFC	SMF
Garuke (G12)	36.7424	7.7281	2017	Ch. 3	SFC	SMF
Garuke (G13)	36.7413	7.7300	2025	Ch. 3,6	SFC	SMF
Garuke (G14)	36.7415	7.7265	2035	Ch. 3	SFC	SMF

Garuke (G15)	36.7399	7.7287	2033	Ch. 3	SFC	SMF
Garuke (G16)	36.7392	7.7311	2028	Ch. 3	SFC	SMF
Garuke (G17)	36.7404	7.7371	2030	Ch. 3	SFC	SMF
Garuke (G18)	36.7405	7.7392	2062	Ch. 3	SFC	SMF
Garuke (G19)	36.7391	7.7385	2051	Ch. 3	SFC	SMF
Garuke (G20)	36.7398	7.7393	2046	Ch. 3	SFC	SMF
Garuke (G21)	36.7384	7.7394	2080	Ch. 3	SFC	SMF
Garuke (G24)	36.7227	7.7256	2062	Ch. 2,3,4,6	SFC	SMF

Appendix Table 2.1 Multiplex panels used for SSR genotyping of *Coffea arabica*

Multiplex panel	Genbank Accession[1]	Dye[2]	PCR primer sequences (5'-3') (F: forward; R: reverse)
A	AJ250253	FAM	F: CTTGTTTGAGTCTGTCGCTG R: TTTCCCTCCCAATGTCTGTA
	AJ250254	FAM	F: GGCTCGAGATATCTGTTTAG R: TTTAATGGGCATAGGGTCC
	AJ308742	VIC	F: GGCTTCTTGGGTGTCTGTGT R: CCATTGGCTTTGTATTTCTGG
	AJ250255	NED	F: CCCTCCCTGCCAGAAGAAGC R: AACCACCGTCCTTTTCCTCG
	AJ250256	PET	F: AGGAGGGAGGTGTGGGTGAAG R: AGGGGAGTGGATAAGAAGG
B	EU526567	FAM	F: CCGACTTGGACTGATGCGAAATTGA R:AAAGCAAAAAACCAGAAAACACGAAGA
	EU526570	VIC	F: CCCCTCCTCCTCCTACTAGATGGTGGT R: GGTCCAGGGTCCATCCATTCTTGA
	EU526586	VIC	F: TGGGTCAAGGATCCGTGTAAGAAAGA R: CCCTCACCAGTTCCCGATGTCAG
	AJ308754	NED	F: TACAAGGGGAGTGGATAAGA

			R: GTTTGTAGGAGGAAGGTGTG
	EU526558	PET	F: CGCGCTTGCTCCCTCTGTCTCT R: TGGGGGAGGGGCGGTGTT
C	EU597609	FAM	F: AGCAACTTCGCCAGTCATTA R: GCGGGTCTTATTCAACGTATAC
	EU597604	VIC	F: CCATTCTAACCAAACCTGTCC R: CTCAAACACTTGGGTGTGCA
	EU597619	VIC	F: CTCTCATCCTTTGCAGCTGA R: TGGGATGCACACTAATCTGC
	AJ308769	PET	F: TCCATCGTTTACGATTTGTC R: GTCATCTATTTGTGAGCTTGG
	EU597601	NED	F: GCATCTTGATTCCCCTTCTC R: GAATAGAGCGAGGCGTGTAT
D	EU597603	FAM	F: TAAAGTGGATGCGTCTCCCA R: GGATAAGCAAGGAGCTGCAA
	EU597615	VIC	F: GAGAGGATCATCGTGATCTTCG R: CCGTCGTTATCTCCTATAAGCC
	EU597627	NED	F: ATGGACAGGAGTTGATGGTACT R: CACTCATTTTGCCAATCTACC
	AJ308776	PET	F: TCTCCCTCTCCCTCTCTCT R: GCGTTTGGTGGAGATGATA
E	EU597612	NED	F: TGGTTGTGCTTACCCTACTAGG

			R: TTGCAAACTTCTCCCGCTAG
	EU597618	VIC	F: TTGCTTGTCTTAGGTAGCCTG
			R: CTAGAAGTGCCAAATGTGAGG
F	AJ250258	FAM	F: AACTCTCCATTCCCGCATTC
			R: CTGGGTTTTCTGTGTTCTCG
	AJ308825	PET	F: TTCTGGTTTCAACTCCATTT
			R: ATAAACCCAAAAAGACCACA
	EU597622	VIC	F: AAGTGCCAAATGTGAGGCGT
			R: AGAAAACACCATCACTCGGT

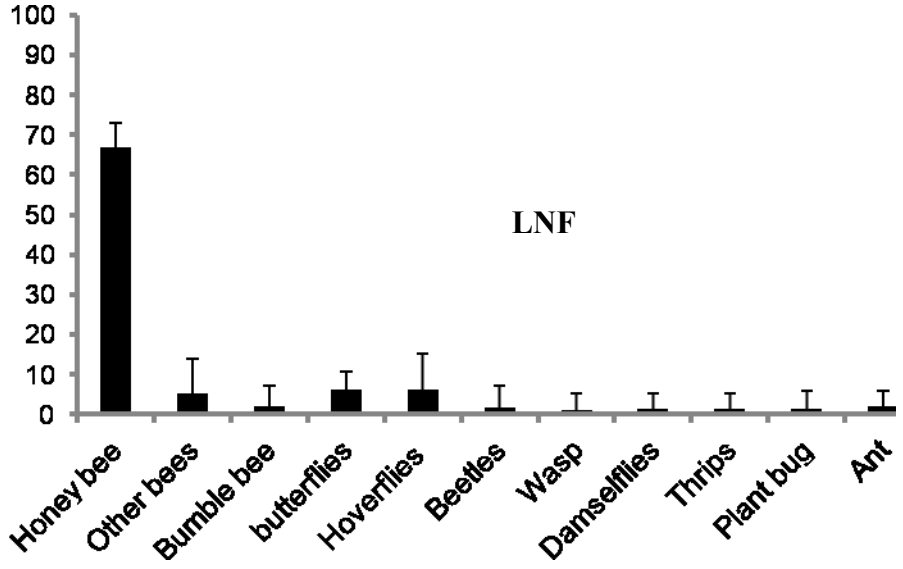
1 GenBank Accession number (nucleotide record accessible via http://www.ncbi.nlm.nih.gov/nuccore/)

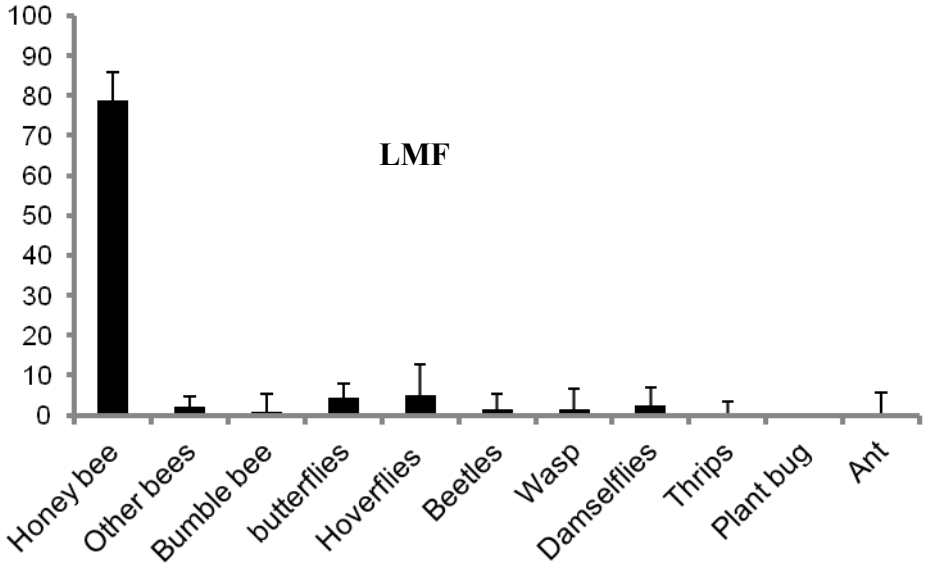
2 DS-33 Applied Biosystems® Standard Dye Set for Genotyping Applications

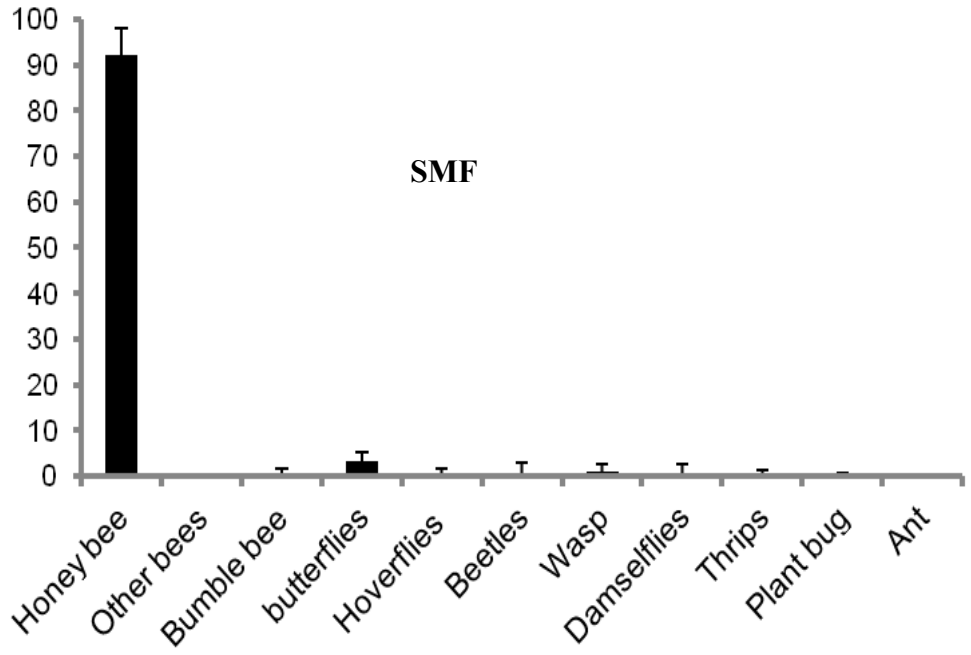
Appendix Table 4.1 List of taxonomic groups of *Coffea arabica* pollinators recorded during observations made along increasing forest fragmentation and management intensity in SW Ethiopian Afromontane coffee forests. SMF: small managed forest fragment; LMF: large managed forest; LNF: large natural forest.

Order/family	Taxonomic groups	LNF	LMF	SMF
Hymenoptera	Honey bee	779	735	729
	Bumble bee	22	7	0
	Other bees	60	20	5
	Wasp	11	13	8
	Ant	22	5	0
Lepidoptera	Butterfly	71	44	28
	Moth	8	1	2
Diptera	Hoverflies	71	50	3
	Common flies	3	2	2
Coleoptera	Beetles	21	16	5
Odonata	Damselflies	17	24	5
Thysanoptera	Thrips	16	4	2
Homoptera	Scale insects/true bugs	22	7	2
Hemiptera	Plant bug	16	0	1
Orthoptera	Grasshopper like spp.	19	12	5
Blatodia	Cockroaches	4	0	0
Total number of individuals netted		1162	970	844

All the netted pollinators were insects (Insecta). The values in the table indicate the abundance of each insect taxonomic group in each forest management type.


LNF
100
90
80
70
60
50
40
30
20
10
0
Honey bee
Other bees
Bumble bee
butterflies
Hoverflies
Beetles
Wasp
Damselflies
Thrips
Plant bug
Ant


LMF
100
90
80
70
60
50
40
30
20
10
0
Honey bee
Other bees
Bumble bee
butterflies
Hoverflies
Beetles
Wasp
Damselflies
Thrips
Plant bug
Ant



Appendix Figure 4.1 Abundance of major taxonomic groups of potential *Coffea arabica* pollinators in three forest management types in southwest Ethiopia. **SMF**: small managed forest; **LMF**: large managed forest; **LNF**: large natural forest. Bars indicate one standard deviation.

Appendix Table 6.1 Factors loading for the soil variables studied

Soil variables	Principal components		
	PC1	**PC2**	**PC3**
pH	0.930	-0.187	-0.062
EC(ds/m)	-0.121	0.514	0.132
Av. B (mg/kg soil)	-0.043	0.557	-0.020
Avl. P(mg/kg soil)	0.365	0.505	0.487
Av. K (mg/kg soil)	0.782	-0.343	0.163
CEC(Cmol(+)/kg soil)	0.054	0.856	-0.286
Ca (Coml.(+)/kg soil)	0.918	0.294	0.068
Mg (Coml.(+)/kg soil)	0.807	-0.007	0.135
Na (Coml.(+)/kg soil)	0.267	-0.088	0.629
K (Coml.(+)/kg soil)	0.818	-0.202	0.170
OC (%)	0.185	0.790	0.412
TN (%)	0.001	0.877	0.168
Mn (mg/kg soil)	-0.554	-0.219	0.098
Fe (mg/kg soil)	0.185	-0.313	-0.745
Cu (mg/kg soil)	0.422	0.606	-0.501
Zn (mg/kg soil)	0.765	0.415	-0.120

Appendix Table 6.2 Means and standard error (S.E.) of the soil variables. The data were organized into two management systems: unmanaged forest coffee (FC) and highly managed semi-forest coffee (SFC).

Soil variables	**FC (n =7)**		**SFC (n = 13**			
	mean	***S.E***	***mean***	***S.E***	***F-value***	***P-value***
pH	5.76	0.24	5.21	0.12	5.47	0.031
EC(ds/m)	0.16	0.02	0.17	0.13	0.31	0.587
Av. B (mg/kg soil)	0.73	0.19	0.71	0.09	0.01	0.931
Avl. P(mg/kg soil)	4.55	1.18	5.02	0.73	0.13	0.724
Av. K (mg/kg soil)	1.23	0.23	0.66	0.08	7.89	0.012
CEC(Cmol(+)/kg soil)	30.08	2.33	38.32	1.93	6.89	0.017
Ca (Coml.(+)/kg soil)	10.12	1.55	8.51	1.01	0.82	0.377
Mg (Coml.(+)/kg soil)	4.76	0.86	3.57	0.40	2.04	0.171
Na (Coml.(+)/kg soil)	0.14	0.02	0.12	0.01	5.56	0.030
K (Coml.(+)/kg soil)	1.18	0.20	0.72	0.08	6.21	0.023
OC (%)	4.73	0.36	5.01	0.28	0.37	0.552
TN (%)	0.45	0.03	0.52	0.02	4.29	0.053
Mn (mg/kg soil)	104.30	17.39	98.32	8.76	0.12	0.734
Fe (mg/kg soil)	89.19	12.97	77.19	7.76	0.72	0.41
Cu (mg/kg soil)	1.09	0.29	2.42	0.36	6.19	0.023
Zn (mg/kg soil)	4.34	0.71	4.34	0.72	0.00	1.000

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